Exhibit 111

Influence of Particle Size on Extrapleural Talc Dissemination After Talc Slurry Pleurodesis*

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Background: **Cases of acute respiratory failure reported after talc pleurodesis have raised concerns about its safety. It has been speculated that this pulmonary inflammatory syndrome is secondary to the extrapleural dissemination of the talc particles.**
Study objectives: **To test the hypothesis that particle size influences extrapleural talc deposition and pleural inflammation after talc slurry pleurodesis.**
Design: **Thirty rabbits underwent pleurodesis as follows: 10 rabbits received 200 mg/kg of the talc used for human pleurodesis, normal talc (NT); 10 rabbits received 200 mg/kg of talc with particles of larger size, large talc (LT); and 10 rabbits received saline solution. Samples from the ipsilateral lung, chest wall, diaphragm, mediastinal pleura, heart, liver, spleen, and right kidney were obtained at 24 h and 7 days and processed for optic and electron microscopy and energy-dispersive x-ray analysis.**
Results: **Visceral pleural thickening was greater with NT than with LT, but no differences were observed in the macroscopic score of adhesions. There was more talc in the lungs of the rabbits that received NT than in those that received LT. Talc particles were detected in mediastinum (100%) and pericardium (20%), irrespective of the talc used. Three animals, all receiving NT, had talc particles in the liver.**
Conclusions: **Our study shows that while both talcs were equally effective in achieving pleurodesis, the intrapleural injection of NT elicits greater pulmonary and systemic talc particle deposition than LT. Moreover, pleural inflammation was greater with NT than with LT.**
(CHEST 2002; 122:1018–1027)

Key words: particle size; pleural; pleurodesis; talc

Abbreviations: Dmax = maximum diameter; EDXA = energy-dispersive x-ray analysis; LT = large talc; NT = normal talc; Sv = surface density; Vn = volume number-weighed; Vv = volume density

Pleurodesis consists of the instillation of a sclerosing agent in the pleural cavity to achieve pleural symphysis. Pleurodesis is indicated for recurrent pneumothorax and for symptomatic relapsing pleural effusion of either malignant or benign etiologies.[1]

Talc is a hydrated magnesium silicate, $Mg_3Si_4O_{10}(OH)_2$. It is one of the most commonly used agents for pleurodesis. The reason for its high popularity is its high effectiveness[2] and low cost. Moreover, a possible therapeutic effect of talc on mesothelioma cells has been suggested.[3]

However, concerns persist as to the development of the ARDS after the intrapleural administration of talc. ARDS has been reported with either insufflated talc powder[4] or talc slurry,[5] and with different talc doses.[6,7] The frequency of ARDS after talc pleurodesis was 3 to 9% in three different series,[6,8,9] and in some cases this complication was lethal.[10] Evidence exists supporting the hypothesis that talc particles instilled into the pleural cavity can escape and migrate to extrapleural organs, thus provoking an inflammatory reaction and acute lung failure. In two studies, talc particles could be detected in the BAL of patients who presented with acute pneumonitis

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Manuscript received July 27, 2001; revision accepted April 3, 2002.
Dr. Ferrer and Dr. Montes contributed equally to the design of the study and writing the article.
Supported by grants from the Fundació Catalana de Pneumologia (Beca Maria Ravà; 1996 FUCAP), Fundación Española de Patología Respiratoria (FEPAR 1997) y Fondo de Investigaciones Sanitarias de la Seguridad Social (FIS-1998).
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after talc pleurodesis.[5,7] In addition, the extrapleural dissemination of talc particles after pleurodesis has been demonstrated in the experimental model. In a study[11] in rabbits, after pleurodesis with talc slurry, talc particles were detected by optic microscopy in 17 to 40% of different extrapleural organs. In a more recent study[12] performed in rats, birefringent particles were found in 100% of extrapleural organs after talc pleurodesis. Talc dissemination can be significant, since lung and hepatic granulomas have been detected after talc was administered by inhalation or IV.[13]

Talc particle size can be a key factor in explaining the extrapleural dissemination of talc from the pleural cavity. Mean particle size among the sterile talcs used for pleurodesis in several countries ranges from 10 to 33 μm, but the lowest mean sizes correspond to the talcs used in the United States.[14] This is remarkable, since most patients who had acute lung disease develop after talc pleurodesis had been treated in the United States.[6,8,9] These facts suggest that particle size can influence the extrapleural dissemination of talc after pleurodesis and may be related to development of acute lung injury.

The aim of the present study was to analyze the extrapleural inorganic deposition and its corresponding histologic lesions after pleurodesis with two talcs of different size distributions. We tested the hypothesis that the smaller the size of the talc particle, the higher the extrapleural deposition of talc and the greater the tissue damage.

Materials and Methods

Talc Preparation and Particle Size Measurement

Two asbestos-free talcs authorized for clinical application were used. Both talcs came from the Respina mine in León, Spain; were produced by Luzenac (Paris, France); and were distributed by Distribuidora de Talco (Distalc; Barcelona, Spain). The talc normally used clinically was called normal talc (NT). Talc with a higher mean particle diameter was called large talc (LT). Spatial characteristics of talc particles were determined in randomly dispersed aerosolized samples of each talc powder before preparing the slurry. Particles were observed by scanning electron microscopy and analyzed by energy-dispersive x-ray analysis (EDXA). Particle size was measured by an automated morphometric and image analysis system, and three-dimensional parameters were estimated by stereology, as described below. Talc was sterilized by autoclaving with an autoclave (Autester-G; Selecta; Barcelona, Spain) at 121°C and 1 atmosphere for 30 min.

General Strategy

Approval for animal experimentation was obtained from the Ethic Committee on Animal Experimentation of the University of Barcelona. Thirty white male New Zealand rabbits weighing 1.5 to 2.0 kg were randomly assigned to the following three experimental groups: NT, LT, and control. Animals from the NT and LT groups received 200 mg/kg of the corresponding talc suspended in 2 mL of saline solution. Control rabbits received only the saline solution. Half of the animals in each group were killed at 24 h, and half were killed 7 days after instillation.

Experimental Procedure

Rabbits were anesthetized with ketamine hydrochloride, 35 mg/kg, and xylazine hydrochloride, 5 mg/kg, administered IM. Under direct view of the parietal pleura, after aseptic surgery talc slurry was instilled into the right pleural cavity with a 27-gauge needle. After suture, animals were turned over to ensure a homogeneous distribution.

The animals were killed with 40 mg/kg of pentobarbital solution injected into the marginal ear vein. The thoracic and abdominal cavities were immediately examined macroscopically. The degree of pleurodesis was graded according to the scheme of Light et al.[15] Samples from lung, chest wall, diaphragm, mediastinal pleura, interpleural adhesions, heart, liver, spleen and right kidney were resected, fixed in 2% paraformaldehyde in 0.1 mol/L phosphate-buffered saline solution (pH 7.4), and processed for optic and electron microscope examination.

Sample Processing

Optical Microscopy: After being fixed by immersion, samples from lung, chest wall, diaphragm, mediastinal pleura, interpleural adhesions, heart, liver, spleen and kidney were dehydrated in solutions with an increasing percentage of ethanol and embedded in paraffin. Samples were then cut with a microtome (model 0325; Anglia Scientific Instruments; Cambridge, UK) to obtain slices at a nominal thickness of 6 μm, which were placed on previously gelatinized slices. Sections were then dewaxed with xylol, hydrated, and stained with Harri's hematoxylin-eosin. Examination was carried out by light field and polarizing microscopy in an optic microscope (Polyvar 2; Reichert-Jung; Vienna, Austria).

Scanning Electron Microscopy: Samples from the lung lower lobe, chest wall, diaphragm, mediastinal pleura, spleen and kidney were cryoprotected by infusion with 30% sucrose in phosphate-buffered saline solution, embedded in optimum-cutting temperature compound (OCT; Miles Laboratories; Naperville, IL), quickly frozen in dry ice and stored at − 35°C. Sections 10 μm thick were cut using a cryostat 2800 Frigocut-E (Reichert-Jung) and spread on stubs covered with poly-l-lysine (Sigma; St Louis, MO). Sections were then washed with double-distilled water, dehydrated, and freeze-dried by the critic point technique and finally recovered with coal. Five slices (lung, chest wall, diaphragm and mediastinal pleura) and 20 slices (spleen and kidney) were prepared for backscattering observation.

Scanning Electron Microscopy and Backscattering Observation and EDXA

Observation based on the retrodispersed electrons, which give a high discrimination power between tissue and talc particles, was carried out with a Cambridge Stereoscan S-120 scanning electron microscope (Cambridge Instruments; Cambridge, UK). Scanning electron microscopy was performed at 20 kV, distance of 21 mm, and angle of 90°. Backscattering observation was carried out with an intensity of 0.9 nanoamperes. The elemental composition of the particles was determined by means of an energy-dispersive x-ray analyzer (Kevex PCXA; LINK; High Wecombe, UK). Elemental silicon (Si) and magnesium (Mg) peaks corresponded to talc.

Visceral Pleural Thickening Estimation

The distance between the pulmonary surface and the underlying parenchyma was measured on hematoxylin-eosin–stained lung sections to quantify the thickening of the visceral pleura. In talc-treated groups, determinations were carried out at a minimum of 5 mm from the focal talc depositions identified in six random selected sections of each rabbit. In the control group, three measurements were obtained in four randomly selected sections of each animal.

Stereologic Estimations

A systematic random strategy was applied as a general rule to sampling blocks, sections, and recounting areas. Quantitative analysis included stereologic parameters as the volume density (Vv) to estimate fractional volumes, mean average volume number-weighted (Vn), and surface density (Sv) to characterize particle populations.[16,17] All light and electron microscopic measurements were carried out by investigators blinded to the category of the rabbit.

Statistical Analysis

All data were expressed as mean ± SEM. Data from estimations were analyzed using a one-way analysis of variance followed by the Fisher exact test. Moreover, specific pairs of estimations were analyzed using two-sample *t* test. Data were considered statistically significant at $p < 0.05$.

Results

Talc Particle Characteristics

Significant differences ($p < 0.001$) were observed between NT and LT particles in all parameters studied (Table 1). Whereas the mean diameter maximum (Dmax) was 8.36 μm (SEM, 0.20) for NT, and 12.00 μm (SEM, 0.25) for LT, the estimated Sv for NT was 33% higher than for LT. In addition, analysis of morphometric and stereologic parameters showed that for equal masses, the number of NT particles was 224% higher than LT, and approximately 300% higher when only particles with a diameter between 0 μm and 10 μm were considered.

Pleural Adhesions

Both types of talc were efficacious in inducing pleural adhesions (Table 2). At 7 days, the mean degree of adhesions for NT and LT was 2.40 (SEM, 0.40) and 2.20 (SEM, 0.49), respectively, which did not differ significantly. In control rabbits, the pleurodesis score was always 0.

Table 1—*Morphometric and Stereologic Parameters of Talc Particles

Variables	Dmax, μm	Vn, μm^2	Sv, $\mu m^2/\mu m^3$
NT	8.36 (0.20)	681.34 (65.85)	1.18 (0.10)
LT	12.00 (0.25)†	1524.11 (101.88)†	0.89 (0.09)†

*Data are presented as mean (SEM).
†$p < 0.001$ compared with NT.

Table 2—*Pleurodesis Score

Groups	24 h	7 d
Control (n = 10)	0	0
NT (n = 10)	1.80 (0.375)	2.40 (0.40)
LT (n = 10)	1.40 (0.245)	2.20 (0.49)

*Data are presented as mean (SEM).

Pleural Inflammation

At both experimental times, and irrespective of the type of talc instilled, talc aggregates ranging from 1 to 12 mm in diameter were found on the pleural surfaces. The largest talc masses were located in small clefts of the pleural surfaces and interlobular spaces, with no regional predominance. Adhesions connecting the visceral and parietal pleura were often associated with these talc accumulations.

By light microscopy, 24 h after talc slurry instillation, NT and LT groups showed focal inflammatory reactions that expanded centrifugally from those points on the pleural surface where talc masses were located (Fig 1, *top left*, *A*). This inflammatory process included both denudement of mesothelium and regression of the basal lamina and underlying connective tissue. Likewise, submesothelial capillary vasodilatation and local endothelial necrosis with extravasation of leukocytes and erythrocytes were observed. In the lung, the inflammation also affected the underlying pulmonary parenchyma, which showed capillary vasodilatation, leukocyte infiltration, and edematous areas (Fig 1, *top right*, *B*, and *center right*, *C*). The extent of inflammation (Table 3) was significantly greater in visceral pleura and subpleural lung of rabbits that underwent pleurodesis with NT (Fig 1, *top right*, *B*) than those treated with LT (Fig 1, *center right*, *C*).

At 24 h, numerous fibrin matrixes were observed associated with the pleural layers (Fig 1, *bottom left*, *D*, and *bottom right*, *E*). These fibrin matrixes contained most of the talc particles instilled, which were evident either as individual particles (Fig 1, *bottom left*, *D*) or massive aggregates (Fig 1, *bottom right*, *E*). Entrapped cells, with the exception of macrophages and some leukocytes, showed regressive characteristics such as apoptotic bodies (Fig 1, *bottom right*, *E*). Focal fibrotic responses developed between the mesothelial and elastic layers between 24 h and 7 days after talc slurry instillation. Consequently, at 7 days, patchy pleural thickening was observed in

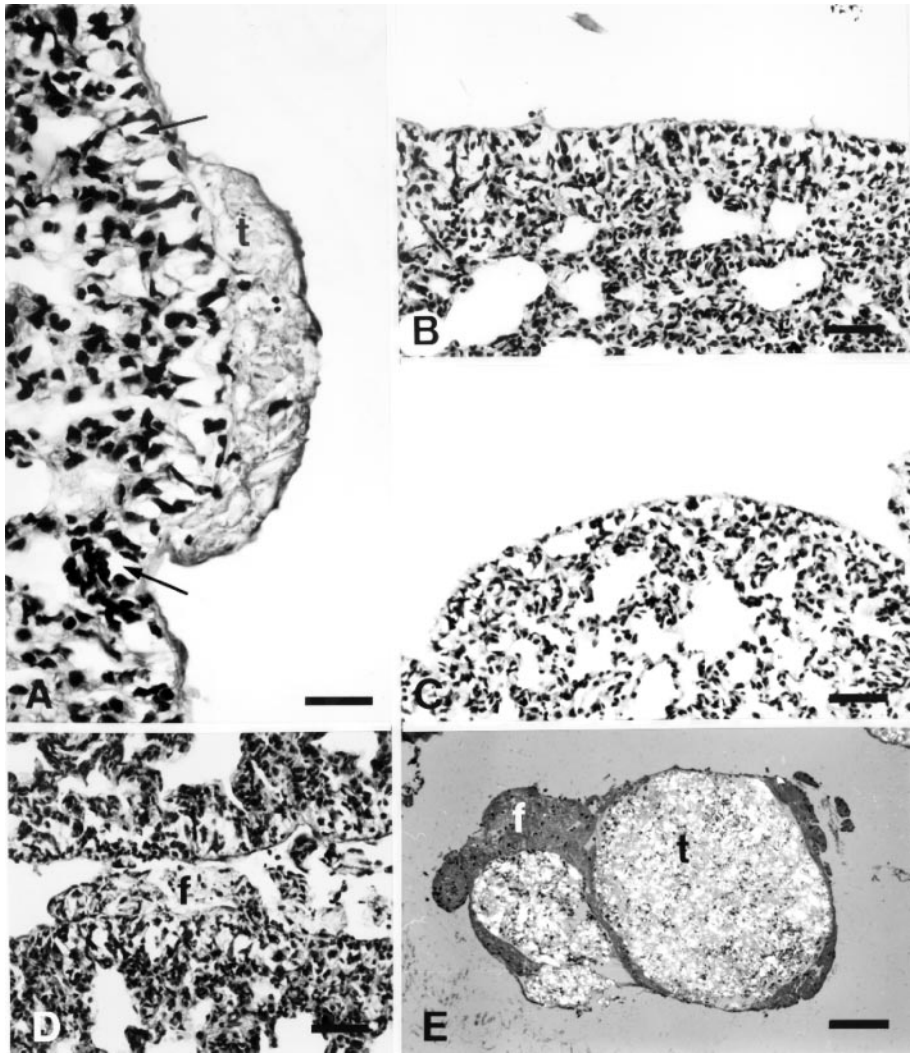

FIGURE 1. Pleural and subpleural inflammation 24 h after talc slurry instillation (hematoxylin-eosin). *Top left*, *A*: talc mass (t) entrapped in a fibrin matrix located at lung surface. The deposition is associated with both mesothelium denudement and subpleural inflammation (arrows) [bar = 25 μm]. *Top right*, *B*, and *center right*, *C*: pulmonary inflammation and leukocyte infiltration (*top right*, *B*, NT group [bar = 50 μm]; *center right*, *C*, LT group [bar = 50 μm]). *Bottom left*, *D*, and *bottom right*, *E*: fibrin matrixes (*bottom left*, *D*, fibrin matrix (f) containing talc particles and extravasated cells located at an interlobular space [bar = 50 μm]; *bottom right*, *E*, massive aggregate of talc particles (t) and cell remnants surrounded by a fibrin matrix (f) at lung surface [bar = 125 μm]).

talc-treated groups, but not in control rabbits. In the lung, the degree of fibrotic pleural thickening (Table 3) was significantly greater ($p < 0.001$) in the NT group (Fig 2, *top left*, *A*) than in the LT group (Fig 2, *top right*, *B*).

At 7 days, light microscopy revealed the presence of both individual particles (Fig 2, *top right*, *B*) and aggregates of talc (Fig 2, *center left*, *C*, and *center right*, *D*) within the thickened submesothelial space. Foreign body granulomas associated with these focal talc depositions were observed (Fig 2, *center left*, *C*, and *center right*, *D*). Although no significant differences were found in the cellular components of the granulomas, they tended to be smaller with LT than with NT.

Between 24 h and 7 days after talc slurry instillation, neovascularization stemming from marginal vessels occurred (Fig 2, *bottom left*, *E*), and fibroblasts, initially located in inflamed submesothelial

Table 3—*Visceral Pleural Thickening

Groups	24 h, μm	7 d, μm
Control	7.8 (1.7)	7.5 (1.6)
NT	63.6 (3.7)†	77.0 (2.8)†
LT	47.9 (5.6)†‡	30.5 (7.3)†‡

*Data are presented as mean (SEM).
†$p < 0.001$ compared with control group.
‡$p < 0.001$ compared with NT group.

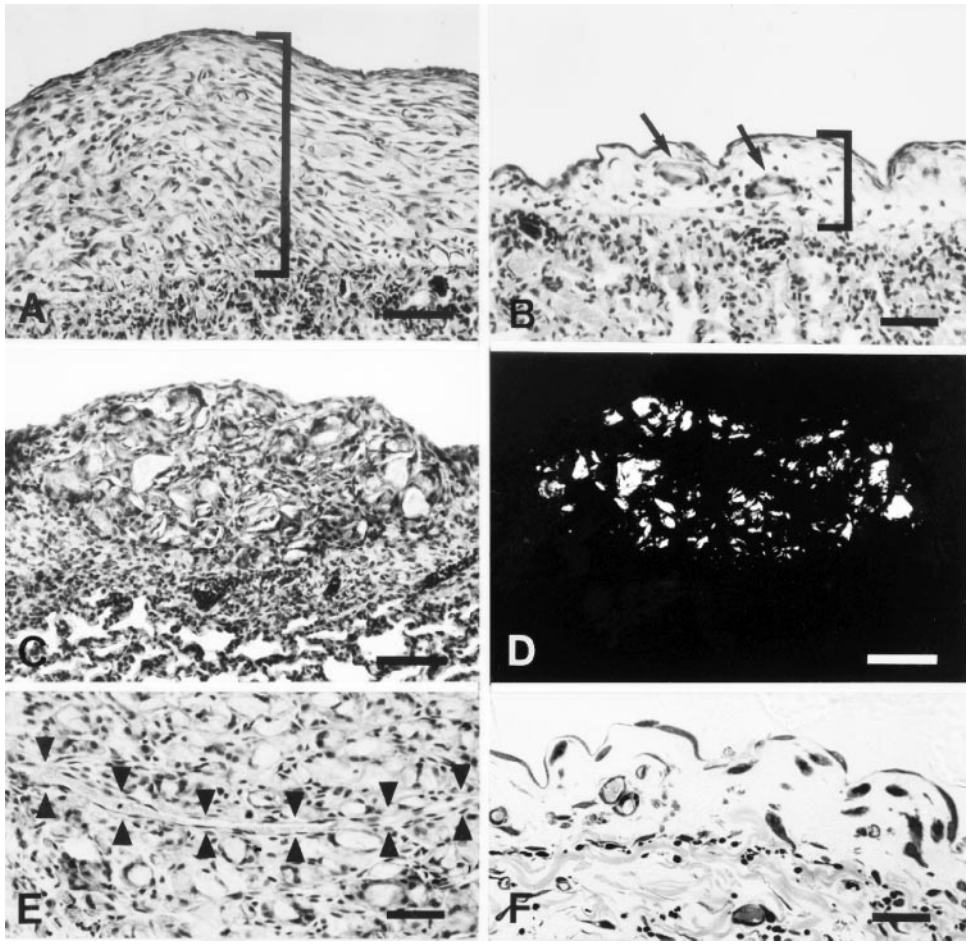

FIGURE 2. Pleural inflammation 7 days after talc slurry instillation (hematoxylin-eosin). *Top left*, *A*, and *top right*, *B*: fibrotic thickening of visceral pleura (square bracket) [*top left*, *A*, NT group (bar = 75 μm); *top right*, *B*, LT group, arrows indicate talc particles (bar = 75 μm)]. *Center left*, *C*, and *center right*, *D*: foreign body granuloma located between the mesothelial and elastic layers in LT group (bars = 75 μm). *Center right*, *D*, is polarized light microscopy view of *center left*, *C*. *Bottom left*, *E*: neovascularization in a granulomatous area; shown is longitudinal section of a new vessel (arrowheads) [bar = 50 μm]. *Bottom right*, *F*: stabilizing repair tissue, newly formed mesothelium covering an adhesion (bar = 25 μm).

areas, were progressively incorporated into fibrin matrixes. At 7 days, fibroblasts were enlarged with strongly stained cytoplasm and lax chromatin, indicating a high protein synthesis rate, with their major axis oriented parallel to collagen fibers (Fig 2, *bottom right*, *F*). These collagen fibers were oriented perpendicularly with reference to the pleural surfaces, and were continuous with the visceral and parietal submesothelial connective tissues. This continuity and the progressive inversion of the cellular fraction to a noncellular component favored the stabilization of the adhesion. Macrophages in the early fibrin matrix persisted in this new scarring tissue, forming both epithelioid and multinucleated giant cells associated with talc particles.

From the first day following talc administration, mesothelial cells located at the margins of denuded areas underwent an active proliferation that re-epithelized all the previously inflamed areas, including fibrotic thickening (Fig 2, *top left*, *A*, and *top right*, *B*), granulomas (Fig 2, *center left*, *C*) and adhesions (Fig 2, *bottom right*, *F*). This newly formed mesothelium was composed of poorly differentiated cells without microvilli. Its basal domain was initially associated with the fibrin matrix, and at 7 days a thin basal lamina was observed underlying the mesothelial cells.

Lung

Several rabbits undergoing talc pleurodesis exhibited talc in the lung. Substantial differences were observed between the experimental groups.

EDXA revealed that 60% (6 of 10 animals) from the NT group showed talc in lung parenchyma. Particle

diameter covered the entire spectrum of the sample. Talc was usually distributed as massive accumulations of particles located mainly in the peripheral parenchyma. The subsequent inflammation of adjacent lung parenchyma resulted in edema and some degree of necrosis. Some contiguous airways, particularly alveoli, alveolar ducts and bronchioles, were disorganized and contained aggregates of particles. No fibroblasts or fibrotic changes were observed related to these particle aggregates. Occasionally, talc followed the bronchovascular spaces and reached small blood and lymphatic vessels, forming small thrombi with blood cells and fibrin (Fig 3).

By EDXA, 20% (2 of 10 rabbits) from the LT group showed talc particles in lung parenchyma. In all cases, the deposition consisted of small particles (Dmax < 10 μm) randomly distributed in the parenchyma. Individual particles were phagocytized by interstitial macrophages, and collections of five or six particles were observed either surrounded by epithelioid cells or inside multinucleated giant cells. Parenchymal fibrosis was not observed (Fig 3).

We defined the following score to semiquantitate the talc deposition in the pleura and lung: I, talc particles incorporated into the pleura but no parenchymal deposition (Fig 3, *top left*, *A*, and *top right*,

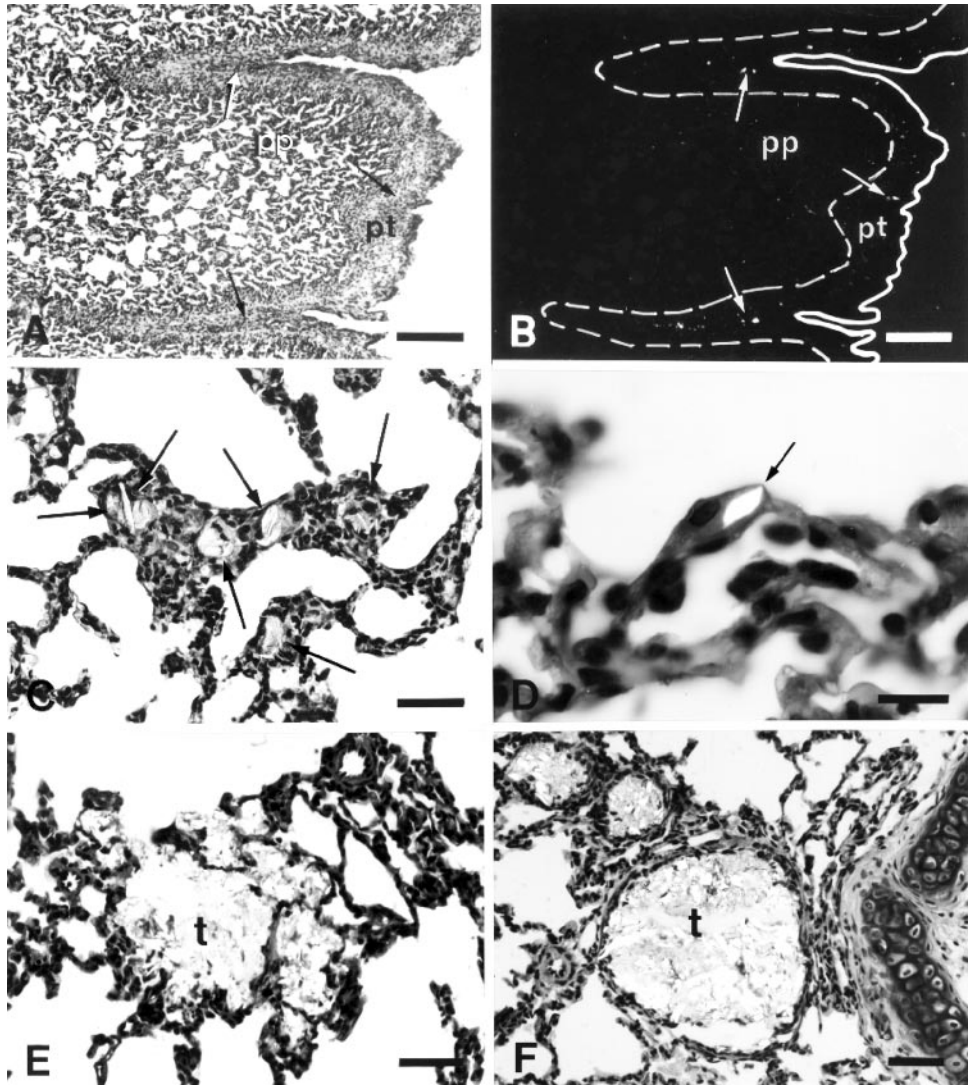

FIGURE 3. Score illustration of pleural and pulmonary deposition of talc (hematoxylin-eosin). *Top left*, *A*, and *top right*, *B*: talc particles (arrows) into the fibrotic submesothelial space after 7 days of LT administration (pp = pulmonary parenchyma; pt = pleural thickening) [bars = 150 μm]. *Top right*, *B*, is a polarization of *top left*, *A*. *Center left*, *C*: individual and small aggregates of talc particles (arrows) located at pulmonary parenchyma after 7 days of NT instillation (bar = 50 μm). *Center right*, *D*: a small talc particle (arrow) inside a parenchymal macrophage from NT group at 7 days (45° polarization; bar = 10 μm). *Bottom left*, *E*: alveoli occupied totally or partially by aggregates of talc particles (t) after 24 h of NT instillation (45° polarization; bar = 50 μm). *Bottom right*, *F*: airways deposition of talc particles (t) from NT group at 24 h (45° polarization; bar = 50 μm).

B); II, individual or small aggregates of talc particles randomly distributed in parenchyma (Fig 3, *center left*, *C*, and *center right*, *D*); and III, diffuse deposition of particles affecting a variable percentage of pulmonary parenchyma (Fig 3, *bottom left*, *E*, and *bottom right*, *F*). According to this score, the intensity of talc particle deposition was greater with NT than with LT, irrespective of the experimental time (Table 4). With NT, five of the six animals showing a pulmonary deposition were classified as stage III, whereas with LT both rabbits with pulmonary deposition were classified only as stage II.

Mediastinum and Mediastinal Pleura

Macroscopically, all talc-treated rabbits showed talc aggregates in the mediastinum with no regional predominance (Table 5). Small talc particles were also observed by polarized light microscopy inside macrophages in the so-called milky spots or Kampmeier foci (Fig 4, *top*, *A*).

Heart and Pericardium

Polarized light microscopy revealed talc particles of variable size in the pericardium and epicardium. Occasionally, talc deposition could be observed at necropsy. Although the number of rabbits affected was greater with NT (3 of 10 rabbits) than with LT (2 of 10 rabbits), differences were not significant (Table 5). The deposition of talc particles in the pericardium was a late phenomenon since, considering both experimental groups, at 24 h only 10% (1 of 10 rabbits) showed talc, whereas at 7 days, the percentage reached 40% (4 of 10 rabbits). The most prevalent histologic changes associated with talc deposition were foreign body granuloma formation and varying degrees of fibrosis (Fig 4, *center*, *B*). In addition, regression and dysplasia of the peripheral muscular cardiac cells was also observed.

Liver

At 7 days, macroscopic examination of the abdominal cavity revealed that three of the five rabbits treated with NT, but none of those receiving LT, showed talc deposition on the liver surface (Table 5).

Table 4—*Score of Talc Deposition in Lung

	NT		LT	
Score	24 h (n = 5)	7 d (n = 5)	24 h (n = 5)	7 d (n = 5)
I	2	2	4	4
II	0	1	1	1
III	3	2	0	0

*Data are presented as No. of rabbits.

Table 5—*Extrapulmonary Talc Deposition

	NT		LT	
Variables	24 h	7 d	24 h	7 d
Mediastinum	5/5	5/5	5/5	5/5
Pericardium	0/5	3/5	1/5	1/5
Liver	0/5	3/5	0/5	0/5
Spleen	1/5	0/5	1/5	0/5
Kidney	1/5	0/5	0/5	0/5

*Data are presented as No. of rabbits/total rabbits.

In one of these animals, a firm symphysis > 2 cm^2 containing a high collection of talc particles was established between the diaphragmatic connective tissue and the Glisson capsule, which became fibrotic and disorganized (Fig 4, *bottom*, *C*). Hepatocytes underlying these talc aggregates became dysplastic. In addition, polarized light microscopy revealed that one rabbit from the NT group, which exhibited the greatest macroscopic deposition of talc, presented small particles (Dmax < 10 μm) inside macrophages located in portal spaces.

Spleen

By EDXA, only two rabbits belonging to the early experimental time showed talc particles in the spleen (Table 5). Whereas one animal from the NT group showed six particles (Fig 5, *top left*, *A*, and *bottom left*, *B*), one rabbit treated with LT had one particle (Fig 5, *top right*, *C*, and *bottom right*, *D*). In both cases, particles were associated with the white periarteriolar substance, and their Dmax were always < 10 μm. Statistically significant ($p < 0.001$) hyperplasia of the white periarteriolar substance was observed at 24 h in all talc-treated animals, but not in control animals (Table 6). At 7 days, the relative volume of the splenic white substance had diminished, but the difference between animals undergoing talc pleurodesis and control animals remained significant ($p < 0.001$).

Kidney

EDXA revealed that only one animal, belonging to the NT group, showed one talc particle (10 μm) in the cortical area of the kidney 24 h after talc administration (Table 5). This animal also had talc particles in the spleen.

Discussion

The results of the present study demonstrate that there is more pulmonary and systemic spread of talc particles with NT than with LT. In addition, there is

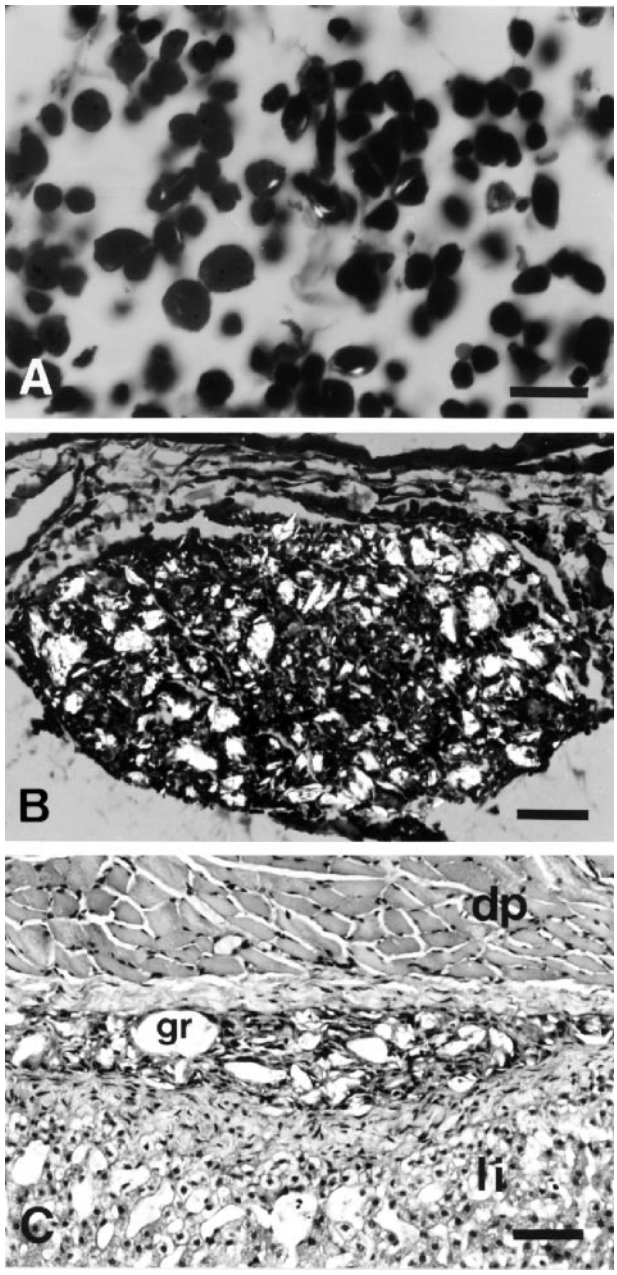

FIGURE 4. Extrapulmonary talc deposition (hematoxylin-eosin). *Top*, *A*: birefringent talc particles inside macrophages from Kampmeier focus in mediastinal pleura (45° polarization from LT group at 7 days; bar = 15 μm). *Center*, *B*: foreign body granuloma located between pericardium and epicardium (45° polarization from NT group at 7 days; bar = 50 μm). *Bottom*, *C*: hepato-diaphragmatic symphysis originated after NT instillation at 7 days (talc particle-containing granuloma [gr] between diaphragm [dp] and liver [li]; bar = 50 μm).

more pleural inflammation and pleural thickening after NT, although the number of adhesions is the same in both groups.

We found that pleurodesis with NT produced greater talc particle deposition in the ipsilateral lung than pleurodesis with LT; more animals were affected with NT, and those that were affected had more talc particles. Our observations suggest that talc reaches the lung parenchyma by breaking the mesothelial and elastic layer. Other proposed escape routes, such as cellular engulfment or intercellular junctions,[18] were not supported by the observations in this study. The fact that the size distribution of talc particles instilled in the pleural cavity and that of NT particles deposited in the lung was the same further supports this mechanism of dissemination.

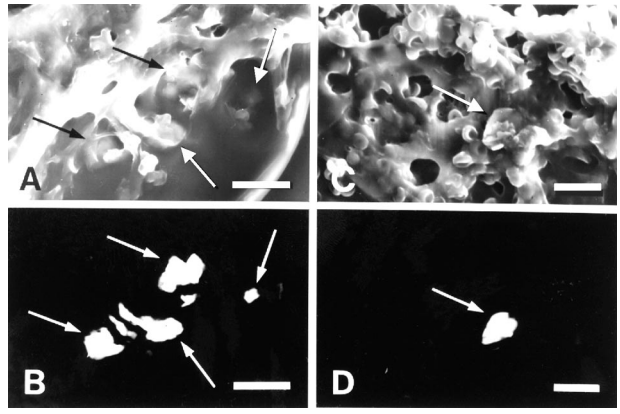

FIGURE 5. Spleen EDXA. *Top left*, *A*, and *bottom left*, *B*: NT group at 24 h (bars = 7 μm). *Top left*, *A*: scanning electron microscopy of talc particles (arrows). *Bottom left*, *B*: backscattering observation of talc particles (arrows). *Top right*, *C*, and *bottom right*, *D*: LT group at 24 h (bars = 7 μm). *Top right*, *C*: scanning electron microscopy of talc particle (arrow). *Bottom right*, *D*: backscattering observation of a talc particle (arrow).

The results of several studies[7,12] suggest that ARDS after talc pleurodesis is due to pulmonary deposition of talc. According to the present results, talc could be found in the lung of any patient undergoing talc pleurodesis, and not only in those who have ARDS develop. However, the fact that only animals treated with NT have diffuse or massive depositions of talc in the lung suggests that the deposition of talc is critically dependent on the size of the particle. If ARDS is due to talc deposition, this provides an explanation for why most patients reported to have this complication received American talc, the particle size of which is the smallest.[14]

A systemic inflammatory reaction seems to develop after talc pleurodesis, as suggested by the fact

Table 6—*Vv of White Pulp vs Spleen**

Groups	24 h, %	7 d, %
Control	14.7 (0.8)	13.7 (0.6)
NT	33.1 (2.7)†	23.2 (1.8)†
LT	35.4 (2.9)†	27.0 (4.1)†

*Data are presented as mean (SEM).
†$p < 0.001$ compared with control group.

that all animals undergoing talc pleurodesis showed hyperplasia of the white periarteriolar substance of the spleen. The finding of Mitchem et al,[19] that rabbits undergoing talc slurry pleurodesis have elevated angiotensin-converting enzyme levels in serum and lung, supports this possibility. However, lung parenchymal changes after talc pleurodesis are basically a granulomatous reaction.

Many talc particles of varying sizes were found in mediastinum in all animals. Some were located in milky spots, thus suggesting that talc, like other particles, is drained from the pleural cavity by the lymphatic system, probably in the parietal pleura.[20] The presence of talc particles in epicardium and pericardium is also remarkable. Particles had different sizes and their number slightly increased between 1 day and 7 days. Talc can reach these structures directly by mechanical progression passing across the mediastinal space. After breaking the pleural mesothelium, talc could progress mechanically and directly penetrate into the pericardial space, which is an alternative pathway to lymphatic dissemination.[18]

Strikingly, three animals undergoing pleurodesis with NT had macroscopically visible talc masses on the liver surface, and one of them had a thick adhesion between the diaphragm and the liver. Since NT produces greater inflammation than LT and a wide range of particle sizes was found in this hepatic adhesion, it can be hypothesized that talc massively leaked from the pleural cavity and reached the diaphragm by mechanical progression through necrotic openings of the damaged mesothelium.

In previous studies, talc particles were detected in abdominal organs after experimental animal pleurodesis. In a study[11] in the rabbit, birefringent bodies were found in abdominal organs in 15 to 40% of the animals studied, but were surprisingly absent in the ipsilateral lung. In a second study[12] in the rat, all extrathoracic organs studied contained birefringent bodies. In the present study, only a few talc particles, all < 10 μm, were observed in the spleen (affecting 2 of 20 animals) and the right kidney (affecting 1 of 20 animals). This was a low-probability phenomenon, probably as a consequence of a passive dissemination via the bloodstream. Furthermore, the low number of affected animals and the rapidity of the process (< 1 day) suggest that particles could have leaked from the pleural cavity into the bloodstream by erosions of the pleural or pulmonary tissue during talc instillation.

There is no doubt that the inflammatory changes observed in the pleura were due to talc, since control animals treated with saline solution showed no such alterations. The main difference between NT and LT was that the intensity of the inflammation was greater with NT, while qualitative aspects were similar. A direct effect of the talc particles on the mesothelium seems to have been the initial mechanism triggering the pleural inflammatory reaction, as suggested by the coincidence of inflammation and talc particle deposition in time and space. Since the pleural cavity is a virtual space, the instillation of talc slurry may place talc particles in rough contact with the mesothelial layer, thus facilitating its direct damage, as proposed for asbestos fibers.[21]

What is the reason for the greater inflammatory power of NT? Since both talcs were asbestos-free and similarly sterilized, the physical characteristics of the particles may be responsible for the differences observed. First, NT, with a lower mean particle size, has a greater number of particles per mass unit. Furthermore, the higher specific surface of NT particles could result in greater cellular damage by particle-dependent mechanisms such as direct injury and oxidative mechanisms.[22] However, cytokine-mediated inflammation may also be related to physical particle characteristics.

High levels of interleukin 8 and monocyte chemoattractant protein 1 are detected in pleural fluid in the first 24 h after human talc pleurodesis,[23] and these cytokines are released by human pleural mesothelial cells after *in vitro* talc stimulation.[24] Several of the changes observed in the present study, such as loss of cellular adhesion, necrosis, and talc phagocytosis, are known to be stimuli for the production of cytokines.[25] The first two are dependent on the particle size and are presumably higher with NT than with LT, as previously discussed. Regarding phagocytosis, we found that only talc particles with a maximal diameter < 10 μm are phagocytized by macrophages, which therefore perhaps explains why NT, with 300% more particles < 10 μm than LT, provokes more phagocytosis-related cytokine release and tissue injury.

The main purpose of pleurodesis is to achieve pleural symphysis and thus prevent further fluid accumulations. In the present study, the constitution of interpleural adhesions was qualitatively similar with both talcs. NT caused greater pleural thickness than LT, but no differences were found in the number of macroscopically visible adhesions or in their microscopic appearance. Thus, the effectiveness of NT and LT as a pleural sclerosing agent appears to be similar.

Some considerations should be made for the results of this study to be extrapolated to clinical practice. First, the talcs used in this study fulfil the requirements for clinical use, and their size is comparable to those used in human pleurodesis. Although the difference in median particle size between NT and LT was not great, a clear difference

existed in size distribution and the percentage of particles < 10 μm. Since the median particle size of talcs from different countries ranges from 10 to 33 μm,[14] differences in extrapleural talc dissemination in human patients could be higher than those observed in this study.

Second, the dose of talc used in this study equaled 12 g in a human patient weighing 60 kg. The reason for administering this high dose was that it is necessary to produce pleural symphysis in rabbits.[15] We do not believe that this high dose was responsible for the extrapleural talc dissemination, since similar or greater talc disseminations were observed after low doses as 60 to 70 mg/kg.[11,12]

Extrapleural talc dissemination has been demonstrated in the present and other animal studies[11,12] and in man.[7] Thus, it seems preferable not to treat patients with benign pleural diseases with intrapleural talc, since the long-term effects of this mineral have not been definitively established. Patients with pleural malignancies are also at risk for ARDS. According to the results of the present study, if talc is chosen as the agent for pleurodesis for those patients, it is probably better to use talc with large particles, since it appears to be equally effective and causes less extrapleural dissemination. The optimum size of talc particles cannot be established from the present data, but it seems advisable to eliminate particles < 10 μm to avoid phagocytosis-related inflammation. Additional studies assessing the safety and effectiveness of pleurodesis with large-particle talc are required to confirm the present data.

ACKNOWLEDGMENT: The authors thank Dr. Charles, Dr. Carreras, Rosa Llòria, and Christine O'Hara.

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Exhibit 112

DOI:10.1093/jnci/djt431

ARTICLE

Aspirin, Nonaspirin Nonsteroidal Anti-inflammatory Drug, and Acetaminophen Use and Risk of Invasive Epithelial Ovarian Cancer: A Pooled Analysis in the Ovarian Cancer Association Consortium

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Manuscript received April 10, 2013; revised November 10, 2013; accepted November 14, 2013.

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Background Regular aspirin use is associated with reduced risk of several malignancies. Epidemiologic studies analyzing aspirin, nonaspirin nonsteroidal anti-inflammatory drug (NSAID), and acetaminophen use and ovarian cancer risk have been inconclusive.

Methods We analyzed pooled data from 12 population-based case–control studies of ovarian cancer, including 7776 case patients and 11 843 control subjects accrued between 1992 and 2007. Odds ratios (ORs) for associations of medication use with invasive epithelial ovarian cancer were estimated in individual studies using logistic regression and combined using random effects meta-analysis. Associations between frequency, dose, and duration of analgesic use and risk of ovarian cancer were also assessed. All statistical tests were two-sided.

Results Aspirin use was associated with a reduced risk of ovarian cancer (OR = 0.91; 95% confidence interval [CI] = 0.84 to 0.99). Results were similar but not statistically significant for nonaspirin NSAIDs, and there was no association with acetaminophen. In seven studies with frequency data, the reduced risk was strongest among daily aspirin users (OR = 0.80; 95% CI = 0.67 to 0.96). In three studies with dose information, the reduced risk was strongest among users of low dose (<100 mg) aspirin (OR = 0.66; 95% CI = 0.53 to 0.83), whereas for nonaspirin NSAIDs, the reduced risk was strongest for high dose (≥500 mg) usage (OR = 0.76; 95% CI = 0.64 to 0.91).

Conclusions Aspirin use was associated with a reduced risk of ovarian cancer, especially among daily users of low-dose aspirin. These findings suggest that the same aspirin regimen proven to protect against cardiovascular events and several cancers could reduce the risk of ovarian cancer 20% to 34% depending on frequency and dose of use.

JNCI J Natl Cancer Inst (2014) 106(2): djt431 doi:10.1093/jnci/djt431

Ovarian cancer is the most fatal gynecologic malignancy, causing more than 140 000 deaths each year worldwide (1). Although early stage ovarian cancer can be successfully treated, the disease is commonly detected at advanced stages with extensive local and systemic spread and poor survival. Early detection strategies have not been shown to reduce mortality (2,3), and biomarker candidates have had insufficient performance to improve early detection efforts thus far (4,5). Primary prevention strategies have not been widely studied but may present alternatives to reduce ovarian cancer burden.

Multiple lines of evidence suggest that ovarian cancer may be related to chronic inflammation (6). In addition to inflammatory factors associated with ovarian epithelial disruption through ovulation (7–9), inflammation-related exposures such as endometriosis (10–12) and exposure to talc or genital powder and asbestos (13) have been associated with increased ovarian cancer risk.

Recently, intervention trials have shown that regular aspirin use is associated with reduced risk of several malignancies (14). However, these trials were not powered for rare cancer endpoints, and none of the clinical trials to date have evaluated ovarian cancer separately. Recent meta-analyses of aspirin use have reached various conclusions that range from no effect (15) to a weak risk reduction among regular users of aspirin (16–18). For nonsteroidal

anti-inflammatory drug (NSAID) use, a recent summary suggested a greater risk reduction among cohort studies than among case–control studies (15), whereas, the results from individual epidemiologic studies have been largely inconclusive (13,19–33), possibly because of limited sample size and limited data on dose, duration, and frequency of use across the studies.

We conducted an analysis of pooled individual-level data of NSAID use and ovarian cancer risk in the Ovarian Cancer Association Consortium (OCAC), including more than 7500 ovarian cancer cases from 12 population-based case–control studies.

Methods

Study Population

We analyzed individual-level data from 12 population-based case–control studies participating in OCAC that had available data on aspirin, nonaspirin NSAID, or acetaminophen (paracetamol) use. All studies had approval from ethics committees, and written informed consent was obtained from study participants. Data acquisition and data pooling for each study were approved by the institutional review board or research ethics committees of the institutes sponsoring the study.

The 12 studies were as follows: the Australian Ovarian Cancer Study and Australian Cancer Study (26), the Connecticut Ovarian Cancer Study (34), the Diseases of the Ovary and their Evaluation Study (23,35), the Hawaii Ovarian Cancer Case–Control Study (36,37), the Hormones and Ovarian Cancer Prediction Study (38), the Malignant Ovarian Cancer Study (39), the North Carolina Ovarian Cancer Study (40,41), the New England Case–Control Study of Ovarian Cancer (42), the New Jersey Ovarian Cancer Study (43), the University of California, Irvine Ovarian Cancer Study (44), the United Kingdom Ovarian Cancer Population Study (45), and the University of Southern California Study of Lifestyle and Women's Health (13) (Table 1). In total, the study included data from nine case–control studies conducted in the United States (13,23,34,37,38,40,42–44), one study conducted in Denmark (39), one study conducted in the United Kingdom (45), and one study conducted in Australia (26).

From these 12 studies, 10 161 ovarian cancer case patients and 12 382 control subjects were available for the analysis. For the primary analysis, we excluded case patients whose cancers were nonepithelial (n = 43), of low malignant potential (n = 2059), or missing data on both the malignant potential of the tumor and tumor grade (n = 68). We further excluded study participants with missing data for all three exposure variables (n = 215 case patients and n=539 control subjects), leaving 7776 invasive ovarian cancer case patients and 11 843 control subjects for our analysis. The case patients were divided into four categories by the four main histologic subtypes of the cancer: serous (n = 4510), endometrioid (n = 1163), clear cell (n = 677), and mucinous (n = 423). The remaining 1003 case patients with cancers of other histologic type were not included in subtype analyses. We also evaluated associations for high-grade serous ovarian tumors (grade II–IV; n = 3786) based on the prevailing view that high-grade serous tumors are distinct from low-grade (grade I; n = 330) serous tumors (46). We evaluated 2059 case patients with cancers of low malignant potential in a separate analysis.

Study Variables

Data for medication use was self-reported in all studies (Table 1). Ten of the 12 studies asked about "regular use" of medications over a specified time period with a minimum frequency of use (13,23,34,38–40,42–45). The duration of regular use varied in the 10 studies, from 1 month to 1 year of use. The majority of the studies, six of 10, specified 6 months or more as the minimum duration (23,38,42–45). The definition for frequency of regular use also varied by study, ranging from once per week to daily; the majority of the studies (n = 8 of 10) specified once or twice per week as the minimum frequency of regular use (13,23,34,38,39,42,44,45). The two remaining studies did not specify regular use, so we reclassified study participants as regular users if their reported frequency of use was at least once per week (26) or if their frequency of use was at least five pills per month and their duration of use was at least 6 months (37).

The exposures used in this analysis were regular (at least once per week) use of aspirin, nonaspirin NSAIDs, and acetaminophen and nonregular use (reference group; less than once a week use for each category). Data for nonaspirin NSAID use were provided in all studies except for two studies that combined aspirin use with other NSAIDs (44,45). Medication use was further classified by frequency [<30 days per month and daily; n = 7 studies (13,23,26,37-40)], dose [<100 and ≥100 mg for aspirin to differentiate between use of low- and regular/high-dose formulations; <500 mg and ≥ 500 mg for non-aspirin NSAID and acetaminophen to differentiate between standard and high-dose formulations; n = studies (37,38,40)], and duration [<60 months and ≥60 months; n = 8 studies (13,23,34,37-39,42,43)] of use based on available data from the individual studies. We created a frequency–dose combination exposure variable based on cross-tabulations of the original categorical variables [(n = 3 studies) (37,38,40)].

Potential confounding variables were available from all studies as part of a core dataset and were harmonized by the coordinating center. Continuous variables were categorized in all analyses for ease of interpretation and to reduce the effect of any outliers. Variables that were selected a priori as adjustment factors included age (5-year categories), race (white, black, other), body mass index (<25, 25–29, ≥30 kg/m^2), use of oral contraceptives (ever, never), parity (nulliparous, 1 full-term birth, >1 full-term birth), menopausal status (pre- or postmenopausal based on study-specific algorithm), and family history of breast or ovarian cancer in a first-degree relative (defined as any breast or ovarian cancer reported in mother, sister, or daughter or breast cancer reported in father). Potential confounding was also evaluated, but not found, for the following variables: Hispanic ethnicity, history of breast feeding, use of estrogen menopausal hormone therapy, use of estrogen plus progestin menopausal hormone therapy, tubal ligation, hysterectomy, and history of endometriosis.

Statistical Analyses

We used multivariable logistic regression models to estimate study-specific odds ratios (ORs) and 95% confidence intervals (CIs) for the association between NSAID exposure and ovarian cancer risk. Study-specific odds ratios were pooled using random-effects meta-analysis to generate a summary odds ratio. For the analyses of the primary exposures (regular use, dose, duration, and frequency), two

Table 1. Characteristics of population-based case–control studies from the Ovarian Cancer Association Consortium included in the pooled analysis*

Study	OCAC acronym	Location	Ascertainment period	Study subjects: Case patients (n = 7776)	Study subjects: Control subjects (n = 11 843)	Question pertaining to drug use	Prevalence of exposure in control subjects: Aspirin %	Prevalence of exposure in control subjects: Nonaspirin NSAID‡ %	Prevalence of exposure in control subjects: Acetaminophen %
Australian Ovarian Cancer Study & Australian Cancer Study† (26)	AUS	Australia	2002–2005	1311	1505	How often have you taken the following over-the-counter (aspirin, paracetamol, anti-inflammatory drugs) medications during PAST 5 years?	10	16	25
Connecticut Ovarian Cancer Study (34)	CON	USA	1999–2003	388	551	Have you ever taken any of the medications shown on this card regularly (at least once per week on average over a duration of 3 months or more)?	26	28	16
Diseases of the Ovary and their Evaluation Study (23,35)	DOV	USA	2002–2009	1159	1849	Before reference date have you taken any of these medications (show card) 5 or more days per month for at least 6 months?	22	27	16
Hawaii Ovarian Cancer Case–Control Study (36,37)	HAW	USA	2001–2008	256	485	Did you ever take an aspirin product (show card) at least 12 times a year? Identical questions ascertained use of acetaminophen (aspirin-free) and NSAIDs.	26	25	22
Hormones and Ovarian Cancer Prediction Study (38)	HOP	USA	2003–2008	683	1513	Prior to reference date have you ever used aspirin (show card) for at least two tablets per week continuously for a period of 6 months or longer? Identical questions ascertained use of over-the-counter pain or inflammation reliever other than aspirin.	34	33	19
Malignant Ovarian Cancer Study (39)	MAL	Denmark	1994–1999	554	1564	Did you ever take medicine on a regular basis, i.e. two times or more per week for more than one month for any of the following conditions?	8	9	5
North Carolina Ovarian Cancer Study (40,41)	NCO	USA	1999–2008	939	1085	For the 5 years prior to diagnosis, did you take any of these over-the-counter medications (show card) on a regular basis for at least 3 months?	11	38	20
New England Case–Control Study of Ovarian Cancer (42)	NEC	USA	1992–2003	870	1243	Prior to reference date have you ever used any over-the-counter pain reliever (show card) continuously at least once a week for a period of 6 months or longer?	18	25	22

(Table continues)

Table 1 (*Continued*).

Study	OCAC acronym	Location	Ascertainment period	Study subjects: Case patients (n = 7776)	Study subjects: Control subjects (n = 11 843)	Question pertaining to drug use	Prevalence of exposure in control subjects: Aspirin %	Nonaspirin NSAID‡ %	Acetaminophen %
New Jersey Ovarian Cancer Study (43)	NJO	USA	2002–2008	238	458	Prior to reference date did you ever take any over-the-counter medications continuously for 6 months or longer (this includes prescriptions, over-the-counter medications, and any natural or alternative treatments you may have taken).	16	9	3
University of California, Irvine Ovarian Cancer Study (44)	UCI	USA	1995–2005	393	313	Have you taken medication listed (aspirin, ibuprofen, acetaminophen, naproxen) regularly? By regular, we are referring to use of the drug or medication at least once a week for a year, or more than 50 pills during a one year-period.	26	41‡	17
United Kingdom Ovarian Cancer Population Study (45)	UKO	UK	2006–2007	516	598	Have you ever used any medication containing the drugs (aspirin, ibuprofen) on a regular basis (by regular we mean every day or almost every day for 6 months or longer)?	15	16‡	—
University of Southern California Study of Lifestyle and Women's Health (13)	USC	USA	2000–2005	469	679	Before reference date, as an adult, did you ever take any prescription or non-prescription medicine at least 2 or more times per week for one month or longer?	15	16	13
						Overall	18	24	16

* NSAID = nonsteroidal antiinflammatory drug; OCAC = Ovarian Cancer Association Consortium.

† Combined for the purpose of this analysis.

‡ UCI and UKO reported data on NSAIDs, including aspirin; the remaining studies provided data on nonaspirin NSAIDs.

multivariable logistic regression models were used: 1) a minimally adjusted model that included covariables for age and race and 2) a fully adjusted model that included age, race, body mass index, oral contraceptive use, parity, menopausal status, and family history of breast or ovarian cancer in a first-degree relative. The summary odds ratios from the fully adjusted model were attenuated slightly compared with the minimally adjusted model. We present the results from the fully adjusted model. We further evaluated models stratified by age (<55 and ≥55 years old), body mass index (<25 and ≥25 kg/m^2), oral contraceptive use (ever/never), and history of endometriosis (yes/no). We assessed asymmetry in study estimates using a funnel plot, and when data were sufficient (n > 5 studies), we formally assessed asymmetry using the adjusted rank correlation (47) and regression asymmetry tests (48). Interstudy heterogeneity was evaluated using I^2.

The following sensitivity analyses were performed: 1) exclusion of tubal or primary peritoneal cases (n = 461); 2) restriction to white non-Hispanic participants because 85% of the participants were of white race and non-Hispanic ethnicity; 3) use of a common reference group analysis, coding "nonregular users" as women who reported no regular use of aspirin or nonaspirin NSAIDs or acetaminophen; 4) restriction of pooled analysis to the six studies that specified 6 months or more as the minimum duration; 5) restriction of pooled analysis to the nine US studies; and 6) exclusion from the pooled analysis the two studies (23,45) with the most restrictive definition of medication use given concerns for misclassification of regular users as unexposed. All statistical tests were two-sided, and P values less than .05 were considered statistically significant. All analyses were performed using STATA software version 11.2 (StataCorp LP, College Station, TX).

Results

Study site, number of case patients and control subjects, and exposure prevalence for each of the 12 OCAC studies are described in Table 1. Overall, 18% of the study population reported regular use (at least once per week) of aspirin, 24% reported regular use of nonaspirin NSAIDs, and 16% reported regular use of acetaminophen.

Aspirin

Figure 1A shows the association between aspirin use (regular vs nonregular use) and ovarian cancer risk. Regular aspirin use was associated with a reduced risk of ovarian cancer (OR = 0.91; 95% CI = 0.84 to 0.99; I^2 = 5.2%). Among seven studies that reported information on frequency of use, daily use was associated with a 20% reduction in ovarian cancer risk (OR = 0.80; 95% CI = 0.67 to 0.96) (Table 2). Among three studies that reported information on dose, low-dose aspirin use (<100 mg/day) was associated with a 34% reduction in ovarian cancer risk (OR = 0.66; 95% CI = 0.53 to 0.83) (Table 2). In analyses of combined categories of frequency and dose of aspirin use, the reduced risk was apparent for daily users of aspirin regardless of dose (low dose: OR = 0.64, 95% CI = 0.50 to 0.81; high dose: OR = 0.78, 95% CI = 0.62 to 0.97) (Table 3).

In subtype analyses, regular aspirin use was associated with reduced risks of serous, endometrioid, and mucinous ovarian cancer, but only the results for serous cancer reached statistical significance (OR = 0.89; 95% CI = 0.80 to 0.99) (Table 4). Pairwise comparisons showed no significant differences in risk between the subtypes (P > .05).

Nonaspirin NSAIDs

Regular nonaspirin NSAID use was associated with a reduced, albeit not statistically significant, risk of ovarian cancer (OR = 0.90; 95% CI = 0.77 to 1.05; I^2 = 73.2%) (Figure 1B). Among the three studies that reported information on dose, high-dose nonaspirin NSAID use (≥500 mg/day) was associated with a 24% reduction in ovarian cancer risk (OR = 0.76; 95% CI = 0.64 to 0.91) (Table 2). In analyses of combined categories of frequency and dose, the reduced risk of ovarian cancer was apparent among both categories of high-dose nonaspirin NSAID use (<30 days per month: OR = 0.77, 95% CI = 0.57 to 1.04; daily: OR = 0.75; 95% CI = 0.60 to 0.94), with a weaker association with daily users of low-dose nonaspirin NSAIDs (OR = 0.88; 95% CI = 0.70 to 1.11) (Table 3). The association between nonaspirin NSAIDs and risk was strongest for serous cancers but did not differ across histologic subtypes of ovarian cancer (Table 4).

Acetaminophen

Acetaminophen use was not associated with ovarian cancer risk (OR = 0.99; 95% CI = 0.88 to 1.12; I^2: 40.0%) (Figure 1C). No associations were observed when analyzing dose, duration, or frequency of acetaminophen use and ovarian cancer risk (Table 2). Further we observed no association between acetaminophen use and histologic subtypes of ovarian cancer (Table 4).

Additional Analyses

The association between NSAID use and high-grade serous tumors was not substantially different than the results reported for all serous tumors combined (results not shown). Tumors of low malignant potential (n = 2059) were not associated with analgesic use (data not shown). In analyses stratified by age, body mass index, oral contraception use, and history of endometriosis, similar NSAID use and ovarian cancer associations were observed as in the overall population (results not shown). Based on the adjusted rank correlation and regression asymmetry tests, there was no indication of small study effects (all P > .05) in the summary estimates for the associations between regular use of aspirin, nonaspirin NSAIDs, or acetaminophen and ovarian cancer. Although there was heterogeneity in the definition of nonaspirin NSAID use, individual exclusion of each study did not substantially change the summary odds ratio (results not shown); however, the exclusion of two studies (13,44) resulted in a decrease in I^2 from 73.2% to 27.8% but no substantial change in the summary odds ratio (results not shown).

In a sensitivity analysis excluding peritoneal and fallopian tube cancers, the pooled summary odds ratios for the associations between regular use of aspirin, nonaspirin NSAIDs, or acetaminophen and ovarian cancer were not substantially different from the odds ratios observed for the overall case group (data not shown). The associations between regular use of NSAIDs and ovarian cancer did not substantially change when the analyses were restricted to non-Hispanic white case patients and control subjects (data not shown). In analyses using women who reported nonregular use of all three NSAIDs as the reference group, a stronger reduced risk was observed for regular use of aspirin (OR = 0.81;

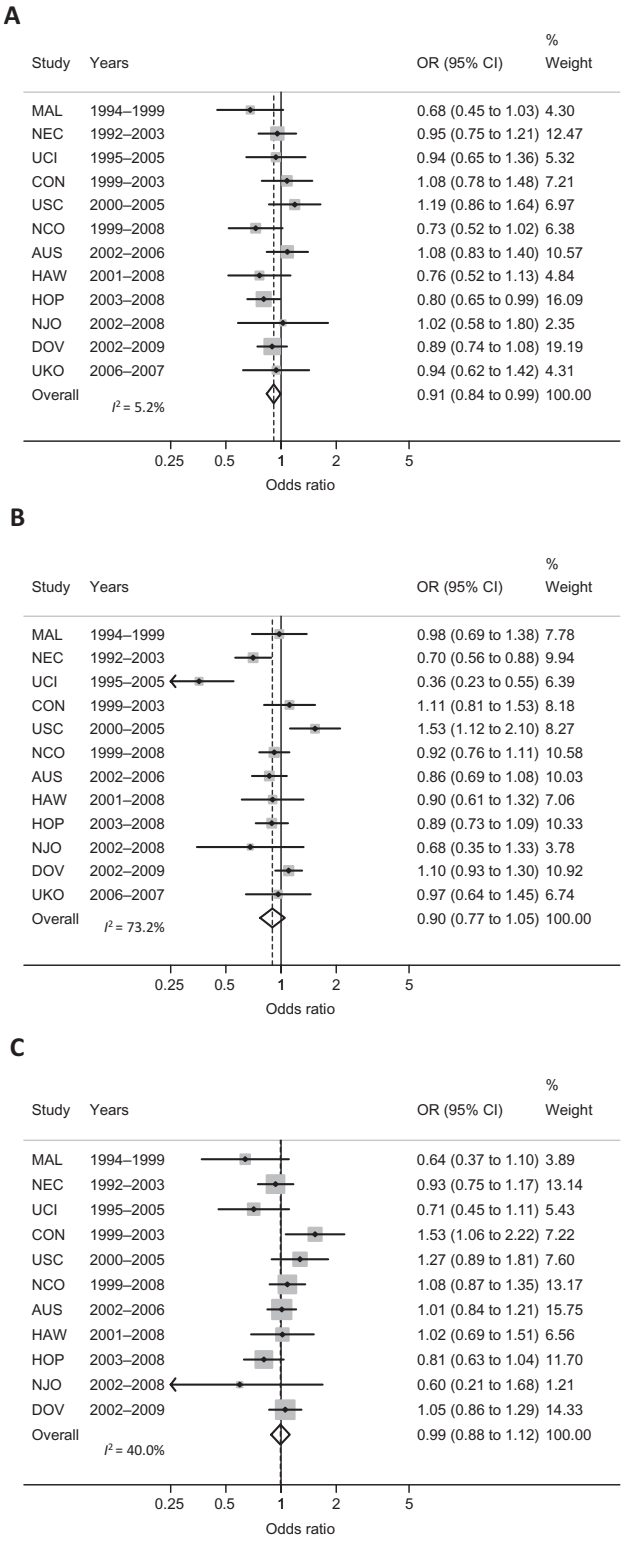

Figure 1. The summary odds ratios (ORs) and 95% confidence intervals (CIs) for the association between regular (at least once per week) use of aspirin (**A**), nonaspirin nonsteroidal anti-inflammatory drugs (NSAIDs) (**B**), and acetaminophen (**C**) and ovarian cancer risk. Summary odds ratios and 95% confidence intervals were estimated using a random-effect meta-analytic model. All statistical tests were two-sided. I^2 is the percentage of variation across studies due to heterogeneity rather than chance. % Weight describes the weight (inverse variance) each study contributed to the summary odds ratio, and the size of the surrounding **square** is an illustrative representation of study weighting. The **horizontal lines** represent study-specific confidence intervals; if ending in an **arrow**, this indicates that the interval transcends the region plotted. The **diamond** represents the summary odds ratio and 95% confidence interval. Studies are presented in order of median year of case accrual from earliest to most recent. AUS = Australian Ovarian Cancer Study, Australian Cancer Study; CON = Connecticut Ovary Study; DOV = Diseases of the Ovary and their Evaluation Study; HAW = Hawaii Ovarian Cancer Study; HOP = Hormones and Ovarian Cancer Prediction Study; MAL = Malignant Ovarian Cancer Study; NCO = North Carolina Ovarian Cancer Study; NEC = New England Case–Control Study of Ovarian Cancer; NJO = New Jersey Ovarian Cancer Study; UCI = University of California, Irvine Ovarian Cancer Study; UKO = United Kingdom Ovarian Cancer Population Study; USC = University of Southern California Study of Lifestyle and Women's Health.

95% CI = 0.68–0.99) and nonaspirin NSAID (OR = 0.86; 95% CI = 0.71–1.05), possibly reflecting reduced "contamination" of the referent group with users of NSAID types other than the medication under examination in each specific analysis (data not shown). In sensitivity analyses restricted to the six studies that specified 6 months or more as the minimum duration or the nine US studies, the pooled summary odds ratios for the associations between regular use of aspirin, nonaspirin NSAIDs, or acetaminophen and ovarian cancer were not substantially different from the odds ratios observed for the overall pooled analysis (data not shown). Finally, in the sensitivity analysis excluding case patients with the most restrictive definition of medication use, the pooled summary odds ratios for the associations between regular use of aspirin, nonaspirin NSAIDs, or acetaminophen and ovarian cancer were not substantially different from the pooled odds ratios observed for all 12 studies (data not shown).

Discussion

To our knowledge, this is the largest evaluation of aspirin, nonaspirin NSAID, and acetaminophen use and ovarian cancer risk to date. We observed a 20% risk reduction for daily users of aspirin and 34% risk reduction for regular users of low-dose aspirin. Regular (at least once per week) use of high doses of nonaspirin NSAIDs was associated with a 24% reduction in ovarian cancer risk. In contrast, acetaminophen use was not associated with ovarian cancer risk. We did not observe any substantial differences in risk by histologic subtypes of ovarian cancer.

Several established risk factors for ovarian cancer are related to inflammatory processes. During ovulation, follicles rupture and inflammatory mediators are released locally that may initiate cell transformation or that may promote growth of transformed cells (49). Proinflammatory agents are also released in inflammatory processes related to endometriosis (10). Aspirin and nonaspirin NSAIDs may reduce exposure to these inflammatory processes; thus, the reduced risk of ovarian cancer with frequent aspirin and nonaspirin NSAID use is consistent with the hypothesized inflammatory etiology of ovarian cancer (50). Several observational studies have evaluated NSAID use and the risk of ovarian cancer. (13,15,19–33,51) A recent meta-analysis reported comparable summary odds ratios for any use of aspirin (OR = 0.91; 95% CI = 0.82 to 1.01) and nonaspirin NSAIDs (OR = 0.89; 95% CI = 0.74 to 1.08), but the estimates did not reach statistical significance (51).

Table 2. Summary odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of aspirin, nonaspirin NSAID, and acetaminophen/paracetamol use with risk of ovarian cancer in the Ovarian Cancer Association Consortium (1992–2009)*

Exposure categorization	Aspirin					Nonaspirin NSAID					Acetaminophen				
	Control	Case	OR†	(95% CI)	I^2‖	Control	Case	OR†	(95% CI)	I^2‖	Control	Case	OR†	(95% CI)	I^2‖
Frequency‡															
No regular use	6366	3826	1.00	(referent)		6007	3565	1.00	(referent)		6189	3497	1.00	(referent)	
<30 days per month	917	739	1.04	(0.92 to 1.18)	0.0	1357	994	1.04	(0.88 to 1.22)	44.8	1805	1439	1.10	(0.96 to 1.26)	0.0
Daily	1179	607	0.80	(0.67 to 0.96)	51.4	1285	776	0.97	(0.83 to 1.12)	46.1	665	427	0.95	(0.74 to 1.23)	63.4
Dose‡§															
No regular use	2138	1359	1.00	(referent)		2053	1274	1.00	(referent)		2465	1516	1.00	(referent)	
Low	320	129	0.66	(0.53 to 0.83)	0.0	439	259	0.96	(0.79 to 1.16)	11.7	113	68	1.15	(0.84 to 1.59)	0.0
High	415	211	0.89	(0.73 to 1.08)	0.0	490	233	0.76	(0.64 to 0.91)	0.0	500	293	0.90	(0.68 to 1.19)	60.4
Duration‡															
No regular use	6625	3667	1.00	(referent)		6451	3568	1.00	(referent)		7106	3918	1.00	(referent)	
<60 months	819	401	0.83	(0.68 to 1.01)	42.3	1002	490	0.86	(0.71 to 1.04)	48.6	477	243	0.88	(0.72 to 1.08)	26.5
≥60 months	833	527	0.98	(0.86 to 1.11)	0.0	824	525	1.08	(0.86 to 1.34)	55.6	712	438	1.13	(0.92 to 1.39)	44.4

* NSAID = nonsteroidal anti-inflammatory drug.

† Summary odds ratios were estimated using random-effects meta-analytic model. Results were adjusted for age (<50, 50–54, 55–59, 60–64, 65–69, ≥70 years), race (white, black, other), oral contraceptive use (ever/never), parity (0, 1, ≥2), menopausal status (premenopausal/postmenopausal), body mass index category (<25, 25–29.9, ≥30 kg/m^2) if available, and first-degree family history of breast cancer, male breast cancer, or ovarian cancer. All statistical tests were two-sided.

‡ Analyses included seven studies for frequency (13,23,26,37–40), three studies for dose (37,38,40), and eight studies for duration.(13,23,34,37–39,42,43).

§ Dose categories for aspirin: low: <100 mg, high: ≥100 mg; for nonaspirin NSAIDs and acetaminophen: low: <500 mg, high: ≥500 mg.

‖ I^2 is the percentage of variation across studies due to heterogeneity rather than chance.

Table 3. Summary odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of aspirin and NSAID use with risk of ovarian cancer in the Ovarian Cancer Association Consortium (1992–2009)*

	Aspirin					Nonaspirin NSAID				
Exposure categorization	**Control**	**Case**	**OR†**	**(95% CI)**	**I^2§**	**Control**	**Case**	**OR†**	**(95% CI)**	**I^2§**
Frequency and dose‡										
No regular use	2138	1359	1.00	(referent)		2053	1274	1.00	(referent)	
<30 days per month, low dose	19	11	1.12	(0.52 to 2.43)	0.0	175	115	1.08	(0.74 to 1.59)	52.1
Daily, low Dose	298	118	0.64	(0.50 to 0.81)	0.0	263	143	0.88	(0.70 to 1.11)	0.0
<30 days per month, high dose	93	66	1.25	(0.88 to 1.76)	0.0	136	82	0.77	(0.57 to 1.04)	0.0
Daily, high Dose	322	144	0.78	(0.62 to 0.97)	0.0	353	148	0.75	(0.60 to 0.94)	3.8

* NSAID = nonsteroidal anti-inflammatory drug.

† Summary odds ratios were estimated using random-effects meta-analytic model. Results were adjusted for age (<50, 50–54, 55–59, 60v64, 65–69, ≥70 years), race (white, black, other), oral contraceptive use (ever/never), parity (0, 1, ≥2), menopausal status (premenopausal/postmenopausal), body mass index category (<25, 25–29.9, ≥30 kg/m^2) if available, and first-degree family history of breast cancer, male breast cancer, or ovarian cancer. All statistical tests were two-sided.

‡ Analyses included three studies for frequency and dose analyses (37,38,40). Dose categories for aspirin: low: <100 mg, high: ≥100 mg; for nonaspirin NSAIDs and acetaminophen: low: <500 mg, high: ≥500 mg.

§ I^2 is the percentage of variation across studies due to heterogeneity rather than chance.

However, daily and/or low-dose aspirin use was not specifically evaluated in the meta-analysis. In contrast, the use of individual-level data in this study facilitated the evaluation of usage patterns beyond what was available in the meta-analysis of published studies.

The pharmacological effects of NSAIDs that lead to reduced risks of cancer or improved cancer prognosis are not well understood and may differ by cancer site. Aspirin is a strong, irreversible inhibitor of COX-1. Nonaspirin NSAIDs are nonselective and reversible inhibitors of both COX-1 and COX-2, whereas acetaminophen is a more effective inhibitor of COX-2 (52,53). The different effects observed in our study for aspirin/nonaspirin NSAIDs and acetaminophen may suggest that COX-1 inhibition is important for ovarian cancer risk reduction, a notion that is further supported by frequent overexpression of COX-1 in ovarian cancer tissue, but more biological and pharmacological research is needed to understand the underlying mechanisms (54).

Both epidemiologic studies and randomized trials have reported inverse associations between aspirin use and colorectal cancer, with a relative risk of approximately 0.5 for regular users (55). There is some evidence that regular and prolonged aspirin use is also associated with reduced risk of cancers of the esophagus (16), bladder (56), liver (57), lung (16), endometrium (58), and female breast (16). A recent pooled analysis of individual patient data from 51 randomized trials of aspirin use for cardiovascular disease prevention reported a 12% reduction in cancer incidence with 3 or more years of daily aspirin use (14). In women, the reduction in incidence was greatest for cancers of the female reproductive organs; however, ovarian cancer incidence was very low (14).

In the Women's Health Study, use of low-dose aspirin every other day was not associated with reduced incidence of colorectal cancer or cancer overall, suggesting that a daily use regimen is important for cancer protection (59). This notion is supported by our findings: the reduction of ovarian cancer risk was much stronger when daily use was considered, and the strongest reduction was observed among daily users of low-dose aspirin. This finding is likely explained by the regular use pattern of low-dose aspirin because low-dose aspirin regimens for cardiovascular protection are characterized by daily use over a long period of time.

Quantifying desired and adverse effects of aspirin will be important when evaluating future public health decisions about aspirin use for prevention of cardiovascular disease and cancer. Complications associated with aspirin use, including peptic ulcer, upper gastrointestinal bleeding, and hemorrhagic stroke, pose serious threats; current risk–benefit analyses favor aspirin use among high-risk groups but not for large-scale, population-based chemoprevention. Our study provides estimates on the effect of aspirin on ovarian cancer risk that should be considered in risk–benefit analyses for preventive aspirin use. However, detailed questions about frequency, dose, and duration will need to be evaluated in future studies including pooled data from cohort studies.

This pooled analysis of data from 12 studies offered several notable strengths. With more than 7500 case patients, we had greater power to detect associations than in any previous single study. Further, we were able to consistently adjust for potential confounders across studies and to evaluate NSAID exposure compared with a common reference group, reducing exposure misclassification (23). Observing consistent associations across studies and countries provided additional robustness to our findings, specifically for aspirin use, where the interstudy heterogeneity was the smallest. The use of individual-level data and the ability to consider and control for a wide range of potential confounders were additional strengths of this pooled analysis.

Potential limitations include possible differential recall of medication use between case patients and control subjects. However, the decreased risk observed for aspirin or nonaspirin NSAIDs and the lack of association with acetaminophen argues against substantial differential recall. Further, the study-specific prevalence of regular aspirin use in the US studies (11%–16%) included in the current analysis is consistent with estimates reported in US cohorts (60–62); differential recall (ie, greater reporting of medication use among case patients) would have biased our results toward the null. There was evidence of heterogeneity between study-specific estimates, but this was mostly restricted to analyses pertaining to nonaspirin NSAIDs and acetaminophen use. Nonaspirin NSAIDs include a variety of drugs and formulations with regional differences that may have contributed to heterogeneity. Another limitation of this pooled analysis was the variability in the definition of regular use across study

Table 4. Summary odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of aspirin, nonaspirin NSAID, and acetaminophen/paracetamol use with risk of ovarian cancer subtype in the Ovarian Cancer Association Consortium (1992–2009)*

Subtype	Aspirin				Nonaspirin NSAID				Acetaminophen			
	Controls	Cases	OR†	(95% CI)	I^2‡	Controls	Cases	OR†	(95% CI)	I^2‡	Controls	Cases	OR†	(95% CI)	I^2‡
Serous															
No regular use	9501	3622	1.00	(referent)		8940	3467	1.00	(referent)		9326	3478	1.00	(referent)	
Use	2123	769	0.89	(0.80 to 0.99)	4.3	2754	1002	0.83	(0.68 to 1.02)	75.4	1878	777	1.03	(0.91 to 1.18)	33.3
Endometrioid															
No regular use	9460	951	1.00	(referent)		8903	858	1.00	(referent)		9264	920	1.00	(referent)	
Use	2115	183	0.90	(0.74 to 1.09)	5.5	2742	290	0.93	(0.75 to 1.15)	38.8	2277	192	0.83	(0.66 to 1.05)	29.1
Clear cell															
No regular use	8800	507	1.00	(referent)		8215	456	1.00	(referent)		9070	510	1.00	(referent)	
Use	1906	110	1.09	(0.84 to 1.41)	9.1	2561	169	0.97	(0.73 to 1.27)	35.0	3222	166	1.22	(0.91 to 1.64)	32.7
Mucinous															
No regular use	8897	308	1.00	(referent)		8340	270	1.00	(referent)		8927	314	1.00	(referent)	
Use	2312	62	0.89	(0.58 to 1.38)	38.1	2625	96	0.99	(0.73 to 1.35)	21.0	1987	66	0.90	(0.66 to 1.23)	0.0

* NSAID = nonsteroidal anti-inflammatory drug.

† Summary odds ratios were estimated using random-effects meta-analytic model. Results were adjusted for age (<50, 50–54, 55–59, 60–64, 65–69, ≥70 years), race (white, black, other), oral contraceptive use (ever/never), parity (0, 1, ≥2), menopausal status (premenopausal/postmenopausal), body mass index category (<25, 25–29.9, ≥30 kg/m^2) if available, and first-degree family history of breast cancer, male breast cancer, or ovarian cancer. All statistical tests were two-sided.

‡ I^2 is the percentage of variation across studies due to heterogeneity rather than chance.

populations. We addressed the misclassification of exposure definitions across the studies by using a standard definition for regular use as described in the Methods; in the two studies with the least restrictive definition of regular use (26,37), participants were reclassified accordingly. We conducted a sensitivity analysis restricting the pooled analysis to those studies with regular use for at least 6 or more months in duration and found similar results. We were not able to reclassify participants from two studies with the most restrictive definition of regular use (23,45). In a sensitivity analysis excluding these two studies from the pooled analysis, the results were essentially unchanged. The details of NSAID use patterns ascertained in each study population differed, and data on frequency, dose, and duration of use were not provided in all studies; thus some subgroup analyses are based on small numbers. Although the point estimates for duration of use suggest a counterintuitive trend of shorter duration of use associated with lower risk of ovarian cancer, the differences were not statistically significant. It will be important to follow up the findings in large pooled prospective studies to better understand the effects of duration and timing of aspirin use and ovarian cancer risk. Further, we were not able to evaluate indication of use.

In summary, this pooled analysis supports the hypothesis that regular aspirin use reduces ovarian cancer risk. Specifically, we report a statistically significant decreased risk of ovarian cancer with daily use of aspirin. Further biological and pharmacological research is necessary to understand the mechanisms of ovarian cancer risk reduction by aspirin use.

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Funding

The Ovarian Cancer Association Consortium was supported by a grant from the Ovarian Cancer Research Fund thanks to donations by the family and friends of Kathryn Sladek Smith. This work was supported in part by the Intramural Research Program of the National Institutes of Health (NIH). Elizabeth Poole and Megan Murphy are both supported in part by training grant T32 CA 09001. Ellen Goode is supported by R01 CA122443 and P50-CA136393. The Australian Ovarian Cancer Study and Australian Cancer Study were funded by the US Army Medical Research and Material Command (DAMD17-01-1-0729), National Health and Medical Research Council of Australia (199600, 400413), Cancer Councils of New South Wales, Victoria, Queensland, South Australia and Tasmania, Cancer Foundation of Western Australia. The Connecticut Ovarian Cancer Study was funded by R01 CA074850 and R01 CA080742. The Diseases of the Ovary and their Evaluation Study was funded by R01 CA112523 and R01 CA87538. The Hawaii Ovarian Cancer Case–Control Study was funded by R01 CA58598, N01 CN55424 and N01 PC67001. The Hormones and Ovarian Cancer Prediction Study was funded by R01 CA95023 and Department of Defense (DOD) grant DAMD17-02-1-0669. The Malignant Ovarian Cancer Study was funded by R01 CA61107, research grant 94 222 52 from the Danish Cancer Society, Copenhagen, Denmark, and the Mermaid I project. The North Carolina Ovarian Cancer Study was funded by R01 CA76016 and DOD grant DAMD17-02-1-0666. The New England Case–Control Study of Ovarian Cancer was funded by R01 CA54419, P50 CA105009, and DOD grant W81XWH-10-1-02802. The New Jersey Ovarian Cancer Study was funded by the National Cancer Institute (K07 CA095666, R01 CA83918, and K22CA138563) and the Cancer Institute of New Jersey. The University of California, Irvine Ovarian Cancer Study was funded by R01 CA58860, R01 CA92044, PSA 042205, and the Lon V Smith Foundation grant LVS-39420. The United Kingdom Ovarian Cancer Population Study was funded by Cancer Research UK, the Eve Appeal and the OAK Foundation. The University of Southern California, Study of Lifestyle and Women's Health was funded by R01 CA17054, R01 CA14089, R01 CA61132, N01-PC-67010, P01 CA17054, California Cancer Research Program (00-01389V-20170, R03 CA113148, R03 CA115195, N01 CN25403), and California Cancer Research Program (2II0200) (USC). The funding agencies did not have any role in conducting the study or preparing the manuscript for publication.

Notes

The Australian Ovarian Cancer Study Management Group (D. Bowtell, G. Chenevix-Trench, A. deFazio, D. Gertig, A. Green, P. Webb) and Australian Cancer Study investigators (A. Green, P. Parsons, N. Hayward, P. Webb, D. Whiteman) thank all the clinical and scientific collaborators (see http://www.aocstudy.org/) and the women for their contribution. The cooperation of the 32 Connecticut hospitals, including Stamford Hospital, in allowing patient access, is gratefully acknowledged. This study was approved by the State of Connecticut Department of Public Health Human Investigation Committee. Certain data used in this study were obtained from the Connecticut Tumor Registry in the Connecticut Department of Public Health. The authors assume full responsibility for analyses and interpretation of these data. The MALOVA group is grateful to Nick Martinussen for data management assistance. The NJO group thanks Drs Lorna Rodriguez and Lisa Paddock, the staff of the New Jersey State Cancer Registry, and Thanusha Puvananayagam for their contribution to the study. Some of this work was undertaken at University College London Hospital/University College London, which received a proportion of funding from the Department of Health's National Institutes for Health Research Biomedical Research Centre funding scheme. We particularly thank I. Jacobs, M. Widschwendter, E. Wozniak, A. Ryan, J. Ford and N. Balogun for their contribution to the study.

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Exhibit 113

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JNCI J Natl Cancer Inst (2019) 111(2): djy100

doi: 10.1093/jnci/djy100
First published online May 31, 2018
Article

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Analgesic Use and Ovarian Cancer Risk: An Analysis in the Ovarian Cancer Cohort Consortium

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Abstract

Background: Aspirin use is associated with reduced risk of several cancers. A pooled analysis of 12 case–control studies showed a 10% decrease in ovarian cancer risk with regular aspirin use, which was stronger for daily and low-dose users. To prospectively investigate associations of analgesic use with ovarian cancer, we analyzed data from 13 studies in the Ovarian Cancer Cohort Consortium (OC3).
Methods: The current study included 758 829 women who at study enrollment self-reported analgesic use, among whom 3514 developed ovarian cancer. Using Cox regression, we assessed associations between frequent medication use and risk of ovarian cancer. Dose and duration were also evaluated. All statistical tests were two-sided.
Results: Women who used aspirin almost daily (≥6 days/wk) vs infrequent/nonuse experienced a 10% reduction in ovarian cancer risk (rate ratio [RR] = 0.90, 95% confidence interval [CI] = 0.82 to 1.00, $P = .05$). Frequent use (≥4 days/wk) of aspirin (RR = 0.95, 95% CI = 0.88 to 1.03), nonaspirin nonsteroidal anti-inflammatory drugs (NSAIDs; RR = 1.00, 95% CI = 0.90 to 1.11), or acetaminophen (RR = 1.05, 95% CI = 0.88 to 1.24) was not associated with risk. Daily acetaminophen use (RR = 1.28, 95% CI = 1.00 to 1.65, $P = .05$) was associated with elevated ovarian cancer risk. Risk estimates for frequent, long-term (10+ years) use of aspirin (RR = 1.15, 95% CI = 0.98 to 1.34) or nonaspirin NSAIDs (RR = 1.19, 95% CI = 0.84 to 1.68) were modestly elevated, although not statistically significantly so.
Conclusions: This large, prospective analysis suggests that women who use aspirin daily have a slightly lower risk of developing ovarian cancer (~10% lower than infrequent/nonuse)—similar to the risk reduction observed in case–control analyses. The observed potential elevated risks for 10+ years of frequent aspirin and NSAID use require further study but could be due to confounding by medical indications for use or variation in drug dosing.

Ovarian cancer is the most fatal gynecologic cancer, largely due to delayed symptom presentation and lack of early detection strategies. Chemoprevention has not been widely studied but may present approaches to reduce ovarian cancer burden. Chronic inflammation likely plays a key role in ovarian carcinogenesis (1). Factors associated with epithelial disruption

Received: January 15, 2018; **Revised:** March 14, 2018; **Accepted:** April 30, 2018

through ovulation (2,3), inflammation-related exposures such as endometriosis and pelvic inflammatory disease (4,5), and circulating biomarkers of inflammation (6,7) have been associated with ovarian cancer risk.

Inhibition of cyclooxygenase (COX) enzymes in prostaglandin synthesis is a primary mechanism responsible for the anti-inflammatory and antineoplastic effects of nonsteroidal anti-inflammatory drugs (NSAIDs) (8,9), and may play a role in ovarian carcinogenesis. Additionally, NSAIDs may suppress ovulation and affect cell proliferation, angiogenesis, and apoptosis of the epithelium (10). Acetaminophen, another common analgesic and antipyretic, has weak anti-inflammatory activity and antigonadotropic effects (11). It also may inhibit ovarian carcinogenesis through the depletion of glutathione leading to necrosis (12). Aspirin, nonaspirin NSAIDs, and acetaminophen are widely used, so any increased or decreased cancer risk may have important public health implications.

Cardiovascular disease prevention trials have shown that daily aspirin use is associated with reduced risk and mortality of several malignancies (eg, colorectal cancer) (13). However, the limited number of women in these trials is insufficient to evaluate ovarian cancer end points (14).

A recent pooled analysis of 12 case–control studies in the Ovarian Cancer Association Consortium (OCAC) reported a reduced risk of ovarian cancer with aspirin use, particularly for daily aspirin users (15). High-dose nonaspirin NSAID use, but not acetaminophen, was also associated with lower risk (15). The few prospective observational studies between aspirin or other NSAID use and ovarian cancer risk have had inconsistent results (16–20). Prospective studies avoid potential biases that may occur in case–control studies, including differences between nonresponders and responders among cases or controls or differences in recollection or reporting of medication use after being diagnosed with ovarian cancer. However, the decreased risk observed for aspirin or nonaspirin NSAIDs and the lack of association with acetaminophen in case–control studies argues against substantial differential recall (15). Further, the exposure window being evaluated in case–control studies is often shortly before cancer diagnosis, during which use may be influenced by preclinical disease. Prospective assessment of analgesic use many years before ovarian cancer diagnosis is necessary to confirm the association with an eye toward improving prevention recommendations. Thus, we evaluated the association between frequent aspirin, nonaspirin NSAID, and acetaminophen use with ovarian cancer risk using prospective individual-level data from the Ovarian Cancer Cohort Consortium (OC3).

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Methods

Study Population

The study population included women participating in 16 prospective cohort studies from North America and Europe (Supplementary Table 1, available online) (16,17,19,21–35). Eligible studies were a cohort study or clinical trial with prospective follow-up including women, determination of ovarian cancer end points through questionnaire/medical record follow-up or confirmation by cancer registries, and follow-up for death. This analysis was limited to 13 studies that collected information on frequent aspirin, nonaspirin NSAID, or acetaminophen (paracetamol) use over at least a six-month period (n = 758 829). All studies obtained institutional approval at their respective institutions; participants provided either written informed consent or implicit consent through return of the study questionnaire. The OC3 Data Coordinating Center and analytic approaches were approved by the institutional review board of the Brigham and Women's Hospital.

Exposure Definitions

Medication use was self-reported at enrollment (Supplementary Table 1, available online) (16,17,19,21,22,24–27,29–34). Given the rationale for assessment of frequent use based on biologic mechanisms and published research (13–15), we focused on frequent medication use (at least 4–5 days/wk) when possible. Frequency was available in 10 of 13 studies (16,17,21,24,25,29–32), whereas three studies included frequency in their definition of regular medication use (19,22,26). Frequent use was defined as use at least four to five times per week for at least six months' duration; less frequent use or nonregular use/no use were combined to form the reference group. We also evaluated very frequent (daily/almost daily) use for at least 6 months' duration as one of the following: six to seven days per week, seven days per week, or 28 or more days per month (11 studies) (16,17,21,22,24,25,29–32). Frequency variables were further divided by duration of use (all medications: ≥0.5–5, >5–10, >10 years, 9 studies) (16,19,24–26,30–32) and aspirin dose (<100 [or "baby aspirin"] and ≥100 mg, four studies) (16,19,23,31).

Potential confounding variables were harmonized from the studies as part of a core data set. A priori adjustment factors included baseline age (continuous), body mass index (<20, 20–24.9, 25–29.9, 30–34.9, ≥35 kg/m^2), number of births (0, 1, 2, 3, ≥4 full-term births), duration of oral contraceptive (OC) use (never, ≤1, >1–5, >5–10, >10 years), and menopause/duration of menopausal hormone therapy (premenopausal, postmenopausal: never, ≤5, >5–10, >10 years).

Outcome Definitions

We included epithelial ovarian or peritoneal tumors identified either through cancer registries or medical record review (ICD9 codes 183 and 158; ICD10 codes C56). We first evaluated associations of medications with all tumors combined (ovarian and peritoneal, n = 3514). Second, we evaluated associations for invasive epithelial ovarian cancers (n = 3147), and, third, we evaluated associations for the four most common tumor histotypes: serous (n = 1475, including tumors coded as poorly differentiated), endometrioid (n = 233), mucinous (n = 125), and clear cell (n = 111). The remaining 1203 cases had another histology (eg, mixed) or were missing histology information (n = 817) and were censored at diagnosis date in histology-specific analyses.

Statistical Methods

Women were excluded from primary analyses if they had a history of cancer (other than nonmelanoma skin cancer) at baseline, bilateral oophorectomy before study entry, or were missing age. We calculated hazard ratios (HRs) and 95% confidence intervals (CIs) using Cox proportional hazards regression to evaluate the association between the analgesic medications and risk of ovarian cancer. Women entered the analysis at age at study entry and contributed person-time until the age at first diagnosis of ovarian cancer (event), death (censored), or end of

follow-up (censored), whichever came first. In primary analyses, we pooled data from all cohorts, stratifying on cohort to account for potential differences in baseline hazards. Secondarily, we used meta-analysis of cohort-specific estimates to assess between-study heterogeneity. Associations between analgesic medication use and ovarian cancer histotype were calculated using competing-risks Cox regression (36). Statistical heterogeneity of associations across histotypes was assessed via likelihood ratio test comparing a model that assumed different associations for the exposure of interest by histotype (full model) with a model with a single estimate across histotypes (reduced model) (37).

Effect modification by factors that influence inflammation (eg, smoking, body mass index [BMI], history of chronic disease) and established ovarian cancer risk factors (eg, age, parity, OC use, endometriosis) was evaluated using multiplicative interaction terms, with statistical significance assessed by a likelihood ratio test.

In sensitivity analyses, we considered a common reference group, coding "nonfrequent users" as women who reported no or infrequent use of aspirin, nonaspirin NSAIDs, and acetaminophen to account for analgesic usage patterns. We also excluded women who reported a history of chronic disease at baseline to assess potential indication for medication use and explored the potential for reverse causation by evaluating associations of frequent analgesic use with ovarian cancer cases that occurred less than five years, five to less than 10 years, and 10 or more years after baseline. Another sensitivity analysis considered death a competing risk (rather than censoring). Exposure curves from survivor function plots were parallel, suggesting no deviation from proportional hazards. All statistical tests were two-sided, and P values of less than .05 were considered statistically significant; analyses were performed using SAS 9.1.

Results

Study Characteristics

The proportion of women reporting frequent analgesic use increased with age; for example, among women reporting frequent aspirin use, 17.7% were younger than age 50 years, whereas 52.2% were 60 years of age or older (Table 1). Compared with women who did not use aspirin or who used it infrequently, women who frequently used aspirin were more likely to be older, be postmenopausal, have a history of a chronic disease, have higher BMI, and were less likely to have previously used OCs. Average follow-up after exposure assessment was 10.8 years (maximum = 18.9 years); individual cohort follow-up is reported in Supplementary Table 1 (available online).

Aspirin

Women who used aspirin at least four to five times per week (n = 851 exposed cases [events]) developed ovarian cancer at about the same rate as women who did not use aspirin or used it only infrequently (HR = 0.95, 95% CI = 0.88 to 1.03) (Table 2). However, compared with infrequent/nonusers, women reporting daily or almost daily use (at least 6 days/wk or more, n = 449 cases) had a 10% reduction in ovarian cancer risk (HR = 0.90, 95% CI = 0.82 to 1.00, P = .05). This association was statistically significant for women reporting daily or almost daily use for 0.5 to less than five years' duration (HR = 0.79, 95% CI = 0.63 to 0.99, P = .04, n = 87 cases) and was suggestively associated for daily users of five to 10 years' duration (HR = 0.88, 95% CI = 0.65 to 1.18, n = 50 cases). Conversely, women who frequently used (vs infrequent/nonuse) aspirin for long durations (≥10 years at baseline) had a non–statistically significantly elevated risk of ovarian cancer (HR = 1.15, 95% CI = 0.98 to 1.34, P =.09, n = 212 cases). No associations were observed when analyzing aspirin dose or other patterns of duration. In analyses by histotype (Table 3), results for serous ovarian cancers were similar to those seen for all ovarian cancer: compared with infrequent/nonuse, daily aspirin use was associated with a 15% decrease for serous tumors (95% CI = 0.71 to 1.00, n = 159 cases), whereas 10 or more years of frequent aspirin use was related to a suggestively elevated risk (HR = 1.27, 95% CI = 0.99 to 1.62, n = 74 cases). A similar pattern was observed for clear cell tumors; however, risk estimates were imprecise due to limited numbers. No associations were observed for endometrioid or mucinous tumors.

Nonaspirin NSAIDs

Women who frequently used nonaspirin NSAIDs had a similar rate of ovarian cancer as infrequent/nonusers (HR = 1.00, 95% CI = 0.90 to 1.11, n = 426 cases) (Table 2). Longer duration or daily frequency of nonaspirin NSAIDs was not related to ovarian cancer risk, although the risk estimate for ovarian cancer for frequent, frequent, long-duration (>10 years) of use of nonaspirin NSAIDs was suggestively elevated (HR = 1.19, 95% CI = 0.84 to 1.68, n = 36 cases). In analyses by histotype, women who frequently used (vs infrequent/nonuse) nonaspirin NSAIDs for long durations had an increased risk of serous tumors than women who used them infrequently or not at all (HR = 2.06, 95% CI = 1.14 to 3.74, n = 10 cases) (Table 3).

Acetaminophen

Frequent use compared with infrequent/nonuse of acetaminophen was not associated with ovarian cancer risk (HR = 1.05, 95% CI = 0.88 to 1.24, n = 152 exposed cases) (Table 2). However, there was a suggestive elevated risk with daily acetaminophen use (HR = 1.28, 95% CI = 1.00 to 1.65, P = .05, n = 71 cases) that was stronger for serous tumors (HR = 1.70, 95% CI = 1.14 to 2.55, n = 26 cases) (Table 3).

Additional Analyses

There was little heterogeneity across studies (data not shown). Risk estimates were generally similar across age strata (Supplementary Table 2, available online). Compared with infrequent/nonusers, daily aspirin use was related to reduced ovarian cancer risk among women younger than age 50 years (HR = 0.89, 95% CI = 0.43 to 1.84), age 50 to 59 years (HR = 0.92, 95% CI = 0.73 to 1.17), and age 60 to 69 years (HR = 0.88, 95% CI = 0.75 to 1.04) at baseline but was null for women age 70 years or older (HR = 1.05, 95% CI = 0.82 to 1.36, $P_{interaction}$ = .73). Daily acetaminophen use was only associated with increased ovarian cancer risk among women age 70 years or older (HR = 1.78, 95% CI = 1.17 to 2.72, $P_{interaction}$ < .001). Results were similar across strata of other ovarian cancer risk factors (data not shown).

Results were similar in analyses restricted to invasive ovarian cancers, utilizing a common reference group, and accounting for death as a competing risk (data not shown). In analyses excluding women with a history of chronic disease, elevated risk estimates with frequent long-duration use of aspirin or

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Table 1. Distribution of frequent analgesic use by baseline demographic and health characteristics in the Ovarian Cancer Cohort Consortium (n = 758 829)

Characteristics	Aspirin		Nonaspirin NSAID		Acetaminophen	
	Infrequent/nonuse No. (%)	Frequent use* No. (%)	Infrequent/nonuse No. (%)	Frequent use* No. (%)	Infrequent/nonuse No. (%)	Frequent use* No. (%)
Age, mean (SD), y	54.7 (11.4)	59.4 (10.1)	59.1 (9.5)	59.6 (8.5)	57.7 (10.6)	60.9 (10.0)
Age, y						
<50	171 049 (31.0)	28 462 (17.7)	68 208 (15.8)	10 496 (12.9)	69 762 (22.9)	3973 (14.4)
50–59	182 326 (33.0)	48 432 (30.1)	144 873 (33.6)	29 425 (36.1)	101 553 (33.4)	8351 (30.3)
60+	198 689 (36.0)	84 044 (52.2)	218 295 (50.6)	41 609 (51.0)	132 697 (43.6)	15 244 (55.3)
BMI, kg/m^2						
<20	38 712 (7.0)	9460 (5.9)	28 981 (6.7)	3239 (4.0)	20 937 (6.9)	1513 (5.5)
20–24.9	246 476 (44.6)	63 791 (39.6)	183 064 (42.4)	25 614 (31.4)	127 806 (42)	9216 (33.4)
25–29.9	157 968 (28.6)	49 716 (30.9)	130 232 (30.2)	25 969 (31.9)	89 960 (29.6)	8560 (31.1)
30–34.9	61 441 (11.1)	21 816 (13.6)	51 919 (12.0)	14 072 (17.3)	36 797 (12.1)	4342 (15.8)
35+	33 201 (6.0)	12 620 (7.8)	26 604 (6.2)	10 813 (13.3)	21 015 (6.9)	3068 (11.1)
Missing	14 266 (2.6)	3535 (2.2)	10 576 (2.5)	1823 (2.2)	7497 (2.5)	869 (3.2)
Age at menarche, y						
≤11	129 521 (23.5)	39 029 (24.3)	104 278 (24.2)	22 428 (27.5)	58 358 (19.2)	5549 (20.1)
12	132 550 (24.0)	43 314 (26.9)	107 177 (24.8)	22 151 (27.2)	82 000 (27.0)	8085 (29.3)
13	155 896 (28.2)	42 510 (26.4)	122 489 (28.4)	19 967 (24.5)	87 684 (28.8)	6628 (24.0)
14	71 928 (13.0)	21 378 (13.3)	55 615 (12.9)	10 314 (12.7)	44 990 (14.8)	4640 (16.8)
≥15	48 479 (8.8)	13 304 (8.3)	38 367 (8.9)	6361 (7.8)	27 904 (9.2)	2428 (8.8)
Missing	13 690 (2.5)	1403 (0.9)	3450 (0.8)	309 (0.4)	3076 (1.0)	238 (0.9)
Duration, oral contraceptive use, y						
Never	210 399 (38.1)	79 036 (49.1)	193 635 (44.9)	32 992 (40.5)	112 760 (37.1)	11 756 (42.6)
>0–1	43 208 (7.8)	14 589 (9.1)	32 672 (7.6)	7606 (9.3)	27 743 (9.1)	2557 (9.3)
>1–5	97 165 (17.6)	24 065 (15.0)	67 121 (15.6)	13 458 (16.5)	47 757 (15.7)	3612 (13.1)
>5–10	78 116 (14.1)	16 254 (10.1)	48 201 (11.2)	9520 (11.7)	36 471 (12.0)	2323 (8.4)
>10	104 143 (18.9)	24 316 (15.1)	76 349 (17.7)	16 530 (20.3)	65 839 (21.7)	6257 (22.7)
Missing	19 033 (3.4)	2678 (1.7)	13 398 (3.1)	1424 (1.7)	13 442 (4.4)	1063 (3.9)
No. of pregnancies						
0	85 920 (15.6)	16 579 (10.3)	56 916 (13.2)	9977 (12.2)	42 630 (14.0)	2899 (10.5)
1	60 572 (11.0)	14 426 (9.0)	45 993 (10.7)	8030 (9.8)	35 178 (11.6)	2988 (10.8)
2	177 064 (32.1)	44 857 (27.9)	128 389 (29.8)	23 169 (28.4)	97 780 (32.2)	7997 (29.0)
3	131 053 (23.7)	42 162 (26.2)	110 188 (25.5)	21 291 (26.1)	67 767 (22.3)	6372 (23.1)
4+	93 130 (16.9)	41 287 (25.7)	85 208 (19.8)	17 992 (22.1)	55 969 (18.4)	6706 (24.3)
Missing	4325 (0.8)	1627 (1.0)	4682 (1.1)	1071 (1.3)	4688 (1.5)	606 (2.2)
Menopausal status						
Premenopausal	188 738 (34.2)	31 168 (19.4)	83 184 (19.3)	12 792 (15.7)	82 248 (27.1)	3986 (14.5)
Postmenopausal	348 494 (63.1)	125 619 (78.1)	342 938 (79.5)	67 335 (82.6)	216 731 (71.3)	22 957 (83.3)
Missing	14 832 (2.7)	4151 (2.6)	5254 (1.2)	5254 (1.7)	5033 (1.7)	625 (2.3)
Age at menopause among postmenopausal women, y						
≤45	45 905 (12.6)	15 523 (12.0)	45 476 (13.1)	8341 (12.1)	33 314 (15.0)	3162 (13.4)
46–50	89 057 (24.5)	32 661 (25.2)	86 398 (24.8)	15 875 (23.1)	60 363 (27.2)	6024 (25.5)
51–55	123 290 (33.9)	43 577 (33.6)	125 242 (36.0)	22 357 (32.5)	77 772 (35.1)	7313 (31.0)
>55	24 452 (6.7)	9294 9294 (7.2)	25 889 (7.4)	5503 (8.0)	14 587 (6.6)	1600 (6.8)
Missing	80 622 (22.2)	28 715 (22.1)	65 187 (18.7)	16 662 (24.2)	35 728 (16.1)	5483 (23.3)
Duration, menopausal hormone use, y						
Never	273 (49.6)	73 279 (45.5)	165 228 (38.3)	26 744 (32.8)	112 911 (37.1)	9282 (33.7)
>0–5	78 (14.3)	29 980 (18.6)	73 431 (17.0)	16 284 (20.0)	54 914 (18.1)	6446 (23.4)
>5–10	43 (7.9)	16 040 (10.0)	41 755 (9.7)	9652 (11.8)	30 399 (10.0)	3512 (12.7)
>10	42 (7.7)	20 700 (12.9)	44 658 (10.4)	13 673 (16.8)	28 174 (9.3)	4487 (16.3)
Missing	113 (20.5)	20 939 (13.0)	106 304 (24.6)	15 177 (18.6)	77 614 (25.5)	3841 (13.9)
History of chronic diseases at baseline included:						
Any cardiovascular disease						
No	19 146 (3.5)	11 630 (7.2)	22 121 (5.1)	8655 (10.6)	26 078 (8.6)	4698 (17.0)
Yes	1763 (0.3)	1545 (1.0)	2500 (0.6)	808 (1.0)	2859 (0.9)	449 (1.6)
Missing	531 155 (96.2)	147 763 (91.8)	406 755 (94.3)	72 067 (88.4)	275 075 (90.5)	22 421 (81.3)
Diabetes						
No	440 316 (85.2)	113 913 (82.0)	308 678 (79.5)	55 152 (81.8)	200 184 (72.8)	14 468 (64.5)
Yes	15 142 (2.9)	9472 (6.8)	16 115 (4.2)	4131 (6.1)	9268 (3.4)	1500 (6.7)
Missing	61 381 (11.9)	15 541 (11.2)	63 500 (16.4)	8161 (12.1)	65 623 (23.9)	6453 (28.8)

(continued)

Table 1. (continued)

Characteristics	Aspirin		Nonaspirin NSAID		Acetaminophen	
	Infrequent/nonuse No. (%)	Frequent use* No. (%)	Infrequent/nonuse No. (%)	Frequent use* No. (%)	Infrequent/nonuse No. (%)	Frequent use* No. (%)
Autoimmune disease						
No	86 690 (18.2)	35 539 (25.5)	104 565 (28.7)	20 401 (31.8)	115 614 (49.5)	9414 (49.4)
Yes	6192 (1.3)	4179 (3.0)	7292 (2.0)	3159 (4.9)	9630 (4.1)	1855 (9.7)
Missing	383 645 (80.5)	99 626 (71.5)	252 748 (69.3)	40 667 (63.3)	108 156 (46.3)	7787 (40.9)

*Frequent: use at least ~4–5 days/wk for 6 months or longer. BMI = body mass index; NSAID = nonsteroidal anti-inflammatory drug.

Table 2. Associations between analgesic use and ovarian cancer risk in the Ovarian Cancer Cohort Consortium (n = 758 829)

Analgesic use	No. of events (cases)	Person-years	HR* (95% CI)	P†
Aspirin				
Infrequent/nonuse	2404	4 946 886	1.00 (ref)	
Frequent use‡	851	1 408 656	0.95 (0.88 to 1.03)	.23
Frequent use by duration vs infrequent/nonuse				
Infrequent/nonuse	1402	3 150 285	1.00 (ref)	
Frequent/0.5–<5 y	239	504 116	0.92 (0.80 to 1.06)	.24
Frequent/5–<10 y	93	171 582	0.90 (0.72 to 1.12)	.33
Frequent/10+ y	212	305 987	1.15 (0.98 to 1.34)	.09
Categories of frequent use vs infrequent/nonuse				
Infrequent/nonuse	1936	3 245 903	1.00 (ref)	
<Daily use	156	161 238	1.06 (0.90 to 1.26)	.49
Daily use§	449	545 499	0.90 (0.82 to 1.00)	.05
Categories of frequent use by duration vs infrequent/nonuse				
Infrequent/nonuse	1402	3 150 285	1.00 (ref)	
<Daily/0.5–<5 y	152	379 640	1.02 (0.85 to 1.21)	.87
<Daily/5–<10 y	43	108 355	0.92 (0.67 to 1.26)	.60
<Daily/10+ y	113	260 503	1.12 (0.92 to 1.37)	.26
Daily/0.5–<5 y	87	124 476	0.79 (0.63 to 0.99)	.04
Daily/5–10 y	50	63 227	0.88 (0.65 to 1.18)	.39
Daily/10+ y	99	45 484	1.18 (0.93 to 1.50)	.18
Frequent use by dose vs infrequent/nonuse				
Infrequent/nonuse	392	436 742	1.00 (ref)	
Frequent low dose	115	72 719	0.99 (0.79 to 1.23)	.91
Frequent normal dose	144	130 684	0.94 (0.77 to 1.15)	.55
Nonaspirin NSAID				
Infrequent/nonuse	2305	3 798 980	1.00 (ref)	
Frequent use‡	426	614 745	1.00 (0.90 to 1.11)	.96
Frequent use by duration vs infrequent/nonuse				
Infrequent/nonuse	1168	2 051 666	1.00 (ref)	
Frequent/0.5–<5 y	122	237 614	0.94 (0.78 to 1.14)	.54
Frequent/5–<10 y	64	75 230	1.10 (0.85 to 1.42)	.49
Frequent/10+ y	36	29 429	1.19 (0.84 to 1.68)	.33
Categories of frequent use vs infrequent/nonuse				
Infrequent/nonuse	1982	3 049 045	1.00 (ref)	
<Daily use	104	124 937	1.07 (0.88 to 1.31)	.50
Daily use§	237	319 625	0.97 (0.84 to 1.11)	.65
Categories of frequent use vs infrequent/nonuse				
Infrequent/nonuse	1168	2 051 666	1.00 (ref)	
<Daily/0.5–<5 y	83	159 749	1.02 (0.81 to 1.28)	.88
<Daily/5–<10 y	39	43 940	1.31 (0.95 to 1.81)	.10
<Daily/10+ y	15	18 356	1.10 (0.66 to 1.84)	.72
Daily/0.5–<5 y	39	77 865	0.81 (0.58 to 1.14)	.23
Daily/5–<10 y	25	31 290	0.86 (0.57 to 1.30)	.48
Daily/10+ y	21	11 074	1.27 (0.80 to 2.01)	.32

(continued)

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Table 2. (continued)

Analgesic use	No. of events (cases)	Person-years	HR* (95% CI)	P†
Acetaminophen				
Infrequent/nonuse	1421	2 583 452	1.00 (ref)	
Frequent use‡	152	213 668	1.05 (0.88 to 1.24)	.61
Frequent use by duration vs infrequent/nonuse				
Infrequent/nonuse	1386	2 425 711	1.00 (ref)	
Frequent/0.5–<5 y	61	95 060	0.99 (0.76 to 1.29)	.93
Frequent/5–<10 y	50	50 683	1.16 (0.87 to 1.54)	.32
Frequent/10+ y	37	51 266	1.01 (0.73 to 1.41)	.96
Categories of frequent use vs infrequent/nonuse				
Infrequent/nonuse	1179	2 120 248	1.00 (ref)	
<Daily use	35	43 645	0.99 (0.70 to 1.39)	.94
Daily use§	71	62 759	1.28 (1.00 to 1.65)	.05
Categories of frequent use by duration vs infrequent/nonuse				
Infrequent/nonuse	1386	2 425 711	1.00 (ref)	
<Daily/0.5–<5 y	33	69 923	0.87 (0.62 to 1.22)	.42
<Daily/5–<10 y	25	35 311	0.98 (0.66 to 1.46)	.93
<Daily/10+ y	22	39 950	0.89 (0.58 to 1.36)	.58
Daily/0.5–<5 y	28	25 137	1.21 (0.81 to 1.81)	.35
Daily/5–<10 y	25	15 372	1.42 (0.94 to 2.13)	.09
Daily/10+ y	15	11 315	1.24 (0.75 to 2.08)	.40

*Hazard ratios and 95% confidence intervals were estimated from Cox proportional hazards models stratified by study cohort and adjusted for baseline age (continuous), body mass index (<20, 20–24.9, 25–29.9, 30–34.9, ≥35 kg/m^2), number of births (none, one, two, three, four or more full–term births), duration of oral contraceptive (OC) use (never, ≤1, >1–5, >5–10, >10 years), and duration of menopausal hormone therapy use (premenopausal, never, ≤5, >5–10, >10 years). CI = confidence interval; HR = hazard ratio; NSAID = nonsteroidal anti-inflammatory drug.

†P value was calculated using a two-sided Wald test.

‡Frequent: use at least ~4–5 days/wk for 6 months or longer.

§Daily: use at least ~6–7 days/wk or ≥28 days per month for 6 months or longer.

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nonaspirin NSAIDs were attenuated (aspirin: HR = 1.11, 95% CI = 0.93 to 1.33; nonaspirin NSAIDs: HR = 1.04, 95% CI = 0.68 to 1.60); other associations, including for acetaminophen, remained unchanged. Associations were slightly stronger for frequent long-duration use of aspirin or daily acetaminophen use for cases diagnosed within five years of baseline compared with five or more years after baseline (data not shown).

Discussion

We observed a 10% reduced ovarian cancer risk for daily aspirin use, although only for women who had used aspirin for less than 10 years; use for 10 or more years was associated with a null or slightly elevated risk. Nonaspirin NSAID and acetaminophen use was not clearly related to ovarian cancer risk overall; however, we observed an increased risk for very frequent (daily/almost daily for at least six months) acetaminophen use. Further, like aspirin, long-duration, frequent nonaspirin NSAID use was associated, at least suggestively, with elevated risk of ovarian cancer. The modestly reduced risk for daily aspirin use is consistent with previous observations from case–control studies (15), although the suggestively elevated risk with long duration of frequent analgesic use requires further evaluation.

Importantly, in this analysis, we were able to evaluate patterns of duration to characterize a dose–response association; however, unlike colorectal cancer, in which longer duration of use is associated with further risk reductions (38), the reduced risk of ovarian cancer with frequent aspirin use was only apparent with short to moderate duration (the largest exposure stratum) and appeared null or slightly elevated with longer-duration use (≥10 years). This may be because those who frequently used aspirin for many years may be more likely to use standard vs low-dose aspirin. That said, availability of data on very long durations of use was limited, as evidenced by the less precise estimates in this group. A better understanding of the relationship between frequency and duration of use leveraging updated exposure data is needed to assess the potential causality of the daily aspirin–ovarian cancer relationship, including ascertainment of use during potentially critical time periods given that the increased risk for long-duration use was strongest for cases diagnosed early in follow-up. Further, consideration of associations for daily aspirin use and its timing/duration with ovarian cancer is needed to fully assess potential for primary prevention, particularly given the relatively low prevalence of ovarian cancer and risk-related adverse events (eg, upper gastrointestinal bleeding). Consistent with our results, pooled analyses of clinical trial data demonstrate that daily aspirin use is most relevant for risk reduction of colorectal cancer and cancer risk overall (39), as alternate dosing trials (higher dose or every other day use) did not show clear benefits (40).

The previous pooled case–control study and our current study support that daily aspirin use is associated with lower ovarian cancer risk. The weaker association in the prospective studies vs case–control studies is similar to results for breast cancer risk (14). Although recall bias may lead to a stronger association in case–control studies, we would expect this to attenuate any true reductions in risk with daily aspirin use. Alternately, considering analgesic use collected at study entry may lead to misclassification of exposure status over follow-up (which averaged more than a decade long) that could attenuate results. Conversely, we observed a consistently elevated ovarian cancer risk with frequent, long-duration use of aspirin and nonaspirin NSAIDs, suggesting potential confounding by medical indications for long-term use. We could not directly address this as indication for use was not collected in most studies. To address this in sensitivity analyses, we excluded women who

Table 3. Associations between analgesic use and ovarian carcinoma histologic subtypes, Ovarian Cancer Cohort Consortium

Analgesic use	P_{het}*	Serous (n = 1470)		Endometrioid (n = 233)		Mucinous (n = 125)		Clear cell (n = 111)	
		No. of events	HR† (95% CI)	No. of events	HR† (95% CI)	No. of events	HR† (95% CI)	No. of events	HR† (95% CI)
Aspirin									
Infrequent/nonuse	.26	1141	1.00 (ref)	181	1.00 (ref)	93	1.00 (ref)	85	1.00 (ref)
Frequent use‡		307	0.93 (0.81 to 1.05)	45	0.90 (0.64 to 1.27)	29	1.13 (0.73 to 1.75)	25	1.11 (0.71 to 1.74)
Frequent use by duration vs infrequent/nonuse									
Infrequent/nonuse	.03	680	1.00 (ref)	132	1.00 (ref)	52	1.00 (ref)	59	1.00 (ref)
Frequent/0.5–<5 y		69	0.85 (0.73 to 0.99)	18	0.93 (0.62 to 1.40)	10	1.03 (0.60 to 1.74)	5	0.75 (0.40 to 1.42)
Frequent/5–<10 y		37	0.89 (0.64 to 1.24)	8	1.28 (0.62 to 2.66)	2	0.67 (0.16 to 2.87)	4	1.46 (0.52 to 4.12)
Frequent/10+ y		74	1.27 (0.99 to 1.62)	8	0.64 (0.31 to 1.31)	10	1.69 (0.83 to 3.42)	10	1.97 (0.98 to 3.97)
Categories of frequent use vs infrequent/nonuse									
Infrequent/nonuse	.13	938	1.00 (ref)	139	1.00 (ref)	62	1.00 (ref)	67	1.00 (ref)
<Daily use		57	1.04 (0.86 to 1.25)	3	0.86 (0.55 to 1.34)	1	0.93 (0.53 to 1.63)	4	1.35 (0.75 to 2.41)
Daily use§		159	0.85 (0.71 to 1.00)	20	0.95 (0.59 to 1.54)	14	1.40 (0.77 to 2.56)	9	0.87 (0.44 to 1.73)
Nonaspirin NSAID									
Infrequent/nonuse	.06	984	1.00 (ref)	139	1.00 (ref)	67	1.00 (ref)	75	1.00 (ref)
Frequent use‡		157	1.09 (0.92 to 1.30)	18	1.03 (0.61 to 1.73)	8	0.86 (0.41 to 1.77)	6	0.53 (0.23 to 1.22)
Frequent use by duration vs infrequent/nonuse									
Infrequent/nonuse	.03	456	1.00 (ref)	71	1.00 (ref)	31	1.00 (ref)	47	1.00 (ref)
Frequent/0.5–<5 y		38	1.01 (0.83 to 1.23)	7	1.09 (0.63 to 1.89)	2	0.84 (0.38 to 1.86)	2	0.54 (0.22 to 1.34)
Frequent/5–<10 y		20	1.39 (0.87 to 2.22)	2	1.04 (0.25 to 4.31)	1	1.51 (0.20 to 11.63)	1	0.71 (0.10 to 4.95)
Frequent/10+ y		10	2.06 (1.14 to 3.74)	0	–	0	–	0	–
Categories of frequent use vs infrequent/nonuse									
Infrequent/nonuse	.04	883	1.00 (ref)	115	1.00 (ref)	61	1.00 (ref)	69	1.00 (ref)
<Daily use		38	1.15 (0.87 to 1.53)	7	1.36 (0.61 to 3.00)	3	1.65 (0.65 to 4.20)	1	0.45 (0.11 to 1.83)
Daily use§		102	1.06 (0.86 to 1.31)	9	0.87 (0.45 to 1.67)	3	0.49 (0.15 to 1.58)	4	0.58 (0.21 to 1.59)
Acetaminophen									
Infrequent/nonuse	.21	577	1.00 (ref)	103	1.00 (ref)	38	1.00 (ref)	50	1.00 (ref)
Frequent use‡		47	1.29 (0.94 to 1.77)	11	1.77 (0.96 to 3.29)	2	0.70 (0.16 to 2.99)	4	1.49 (0.43 to 5.17)
Frequent use by duration vs infrequent/nonuse									
Infrequent/nonuse	.01	557	1.00 (ref)	100	1.00 (ref)	38	1.00 (ref)	46	1.00 (ref)
Frequent/0.5–<5 y		22	1.36 (0.87 to 2.12)	3	0.72 (0.20 to 2.64)	0	–	3	2.42 (0.57 to 10.35)
Frequent/5–<10 y		15	1.44 (0.85 to 2.43)	5	3.66 (1.54 to 8.69)	1	1.68 (0.23 to 12.17)	1	1.48 (0.18 to 11.91)
Frequent/10+ y		8	0.97 (0.48 to 1.96)	3	1.92 (0.58 to 6.32)	1	1.30 (0.16 to 10.48)	0	–
Categories of frequent use vs infrequent/nonuse									
Infrequent/nonuse	.09	554	1.00 (ref)	102	1.00 (ref)	35	1.00 (ref)	46	1.00 (ref)
<Daily use		9	0.95 (0.60 to 1.51)	1	1.70 (0.78 to 3.69)	1	1.15 (0.22 to 6.03)	3	1.69 (0.33 to 8.59)
Daily use§		26	1.70 (1.14 to 2.55)	6	1.85 (0.75 to 4.57)	0	–	1	1.15 (0.17 to 8.01)

*The $P_{heterogeneity}$ value was calculated using a two-sided likelihood ratio test (37). CI = confidence interval; HR = hazard ratio; NSAID = nonsteroidal anti-inflammatory drug.

†Hazard ratios and 95% confidence intervals were estimated from competing risk (37). Cox proportional hazards models were stratified on study cohort and adjusted for baseline age (continuous), body mass index (<20, 20–24.9, 25–29.9, 30–34.9, ≥35 kg/m^2), number of births (none, one, two, three, four, or more full-term births), duration of oral contraceptive (OC) use (never, ≤1, >1–5, >5–10, >10 years), and duration of menopausal hormone therapy use (premenopausal, never, ≤5, >5–10, >10 years). Competing risk models were based on fixed covariate effects; variable covariate effect results were practically identical (data not shown).

‡Frequent: use at least ~4–5 days/wk for 6 months or longer.

§Daily: use at least ~6–7 days/wk or ≥28 days per month for 6 months or longer.

reported a history of chronic disease at baseline and observed some attenuation in risk estimates. That said, further assessment of confounding by medical indications for long-term use, such as joint pain, osteoarthritis, cardiovascular disease, or other factors, is needed, as well as consideration of potential biologic mechanisms by which long-term use may increase risk.

Consistent with our results, acetaminophen use was not associated with ovarian cancer risk in the pooled case–control study data (15), based on more than 400 exposed cases (odds ratio for daily vs nonregular use = 0.95, 95% CI = 0.74 to 1.23). Acetaminophen and nonaspirin NSAIDs are commonly used interchangeably; however, acetaminophen has weak anti-inflammatory properties and may have gonadotrophic effects (11), supporting the different associations we observed between NSAIDs and acetaminophen in our study and suggesting different anti-inflammatory effects or other mechanisms of action (8,9,11). Importantly, the increased risk with daily acetaminophen use observed in this study was based on a limited number of exposed cases and should be interpreted with caution.

The consistent positive relationship for frequent long-duration use of aspirin or nonaspirin NSAIDs with serous disease may suggest that long-term users likely have other factors that increase inflammation and thus risk of this histotype. Some data suggest that serous tumors may be more strongly related to inflammatory factors. For example, aggressive high-grade serous tumors have been more commonly associated with inducible nitric oxide synthase and other inflammatory markers than low-grade tumors (41). Further, prediagnostic circulating inflammatory marker, C-reactive protein, has been associated with the serous histotype (6,42). Lifetime ovulations also were more strongly associated with tumors expressing p53 (43), a hallmark of serous disease (44).

The prospective design of the pooled studies precludes recall bias. Additional strengths of the study include the large sample size, the ability to identify deaths as well as capture loss to follow-up, and the ability to account for many known and suspected risk factors for ovarian cancer. Limitations included the use of self-reported exposure data, limited information on low-dose aspirin use, and limited data on health conditions or medical indications underlying long-term analgesic use. The combination of long-term follow-up and ascertainment of exposure at baseline (in most studies) mean that individuals could have started or stopped use during follow-up, which would contribute to measurement error. Further, information on duration of use at baseline may not adequately represent exposure duration, as such confounding by indication may not fully explain the elevated risks. Residual confounding by age-related factors may also be present; however, we did not observe substantial differences in associations across age strata.

The incidence of ovarian cancer is low; thus, our modest findings are unlikely to alter the balance of more common and clinically significant risks and benefits associated with daily aspirin use. However, the associations stratified by age at baseline provide information relevant to current US Preventive Services Task Force recommendations regarding aspirin use for cardiovascular prevention (45), as decreased ovarian cancer risk estimates associated with daily aspirin use were only observed among women younger than age 70 years. The USPSTF does not recommend frequent aspirin use in women age 70 years or older because of increased risk for adverse events. Although the potential increased risk associated with daily acetaminophen and frequent aspirin and nonaspirin NSAID use for more than 10 years' duration requires further study, daily aspirin use may provide a very modestly reduced risk with respect to incident ovarian cancer.

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Funding

This work was supported by Department of Defense Ovarian Cancer Research Program grant W81XWH-12-1-0561. The UKBGS thanks Breast Cancer Now and the Institute of Cancer Research (ICR) for support and funding. The ICR acknowledges National Health Service funding to the National Institute for Health Research Biomedical Research Centre. K05CA154337 from the National Cancer Institute (NCI) and Office of Dietary Supplements (VITAL); R01 CA39742 (Iowa Women's Health Study); research grants from the Swedish Research Council and Swedish Cancer Foundation (SMC, WLHS); UM1 CA164973 (Multiethnic Cohort Study [MEC]); NIH/NCI UM1 CA182876 (SCHS); UM1 CA186107, P01 CA87969, UM1 CA176726, R01 CA67262 (Nurses' Health Study, Nurses' Health Study II); and NIEHS Intramural Research Program (Project Z01-ES044005 to DPS). The Womens Health Initiative (WHI) program is funded by the National Heart, Lung, and Blood Institute, NIH/DHHS, through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. NCI Intramural Research Program.

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The funding agency did not have any role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

Preliminary results from this study were presented at the AACR Rivkin Ovarian Cancer Meeting (September 2016) and at the NCI Cohort Consortium Annual Meeting (November 2016, same abstract).

The authors thank Ruifeng Li for assistance with computer programming and harmonization of covariate data. The UKBGS thanks the study participants, study staff, and the doctors, nurses, and other health care staff and data providers who contributed to the study. We would like to thank the participants and staff of the NHS/NHSII for their valuable contributions and the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. The NLCS thanks participants and staff who have contributed to the study. The authors thank the WHI investigators for their dedication and the WHI study participants for making the program possible.

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Exhibit 114

FULL PAPER

BJC

British Journal of Cancer (2016) 114, 819–825 | doi: 10.1038/bjc.2016.39

Keywords: ovarian cancer; African American; analgesics; aspirin; non-steroidal anti-inflammatory drugs; acetaminophen

Analgesic medication use and risk of epithelial ovarian cancer in African American women

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Background: Existing literature examining analgesic medication use and epithelial ovarian cancer (EOC) risk has been inconsistent, with the majority of studies reporting an inverse association. Race-specific effects of this relationship have not been adequately addressed.

Methods: Utilising data from the largest population-based case–control study of EOC in African Americans, the African American Cancer Epidemiology Study, the relationship between analgesic use (aspirin, non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), and acetaminophen) and risk of EOC was estimated by multivariate logistic regression. The association of frequency, duration, and indication of analgesic use on EOC risk was also assessed.

Results: Aspirin use, overall, was associated with a 44% lower EOC risk (OR = 0.56; 95% CI = 0.35–0.92) and a 26% lower EOC risk was observed for non-aspirin NSAID use (OR = 0.74; 95% CI = 0.52–1.05). The inverse association was strongest for women taking aspirin to prevent cardiovascular disease and women taking non-aspirin NSAIDs for arthritis. Significantly decreased EOC risks were observed for low-dose aspirin use, daily aspirin use, aspirin use for <5 years, and occasional non-aspirin NSAID use for a duration of $\geqslant 5$ years. No association was observed for acetaminophen use.

Conclusions: Collectively, these findings support previous evidence that any NSAID use is inversely associated with EOC risk.

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Received 9 November 2015; revised 21 January 2016; accepted 28 January 2016; published online 23 February 2016

Inflammation may play a role in ovarian cancer carcinogenesis through the production of toxic oxidants and bioactive substances, increasing the chances of DNA damage and mutagenesis (Ness and Cottreau, 1999). Analgesic drugs, such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), have anti-inflammatory properties and have been associated with reduced risks of several malignancies (Schreinemachers and Everson, 1994; García-Rodríguez and Huerta-Alvarez, 2001; Bosetti *et al*, 2012; Rothwell *et al*, 2012; Neill *et al*, 2013). Another commonly used type of analgesic medication, acetaminophen, has weak anti-inflammatory activity, but may reduce cancer risk through antigonadotropic effects (Cramer *et al*, 1998) that may be particularly relevant to ovarian cancer.

The existing literature examining analgesic drug use and ovarian cancer risk is inconsistent, with the majority of studies reporting mild protective associations (Rosenberg *et al*, 2000; Schildkraut *et al*, 2006; Wernli *et al*, 2008; Pinheiro *et al*, 2009) or no association (Moysich *et al*, 2001; Lacey *et al*, 2004; Murphy *et al*, 2012), with few suggesting weak positive relationships (Hannibal *et al*, 2008; Wu *et al*, 2009). A meta-analysis of 17 studies concluded that the existing body of evidence does not clearly support the presence of an association between analgesic use and ovarian cancer risk (Ni *et al*, 2013). However, a recent, well-powered pooled analysis using data from 12 case–control studies participating in the Ovarian Cancer Association Consortium observed a statistically significant decrease in epithelial ovarian cancer (EOC) risk for aspirin use, a decrease in risk for high-dose non-aspirin NSAID use, and no association for acetaminophen use (Trabert *et al*, 2014).

The majority of published literature examining the relationship between analgesic use and ovarian cancer was conducted in study populations composed predominately of white women, with little representation of African American (AA) women. There are several indications that a differing risk profile may be evident by race; published studies have suggested that AA women have higher inflammatory marker levels (e.g., interleukin-6, C-reactive protein) than white women (Albert *et al*, 2004; Khera *et al*, 2005; Paalani *et al*, 2011) and that there are differences in patterns of analgesic use by race (Zhou *et al*, 2014). To our knowledge, only one study (Setiawan *et al*, 2012) has evaluated race-specific associations for analgesic use and risk of EOC. Using the Multiethnic Cohort Study, Setiawan *et al* (2012) reported a weak inverse association, although not significant, between analgesic drug use and EOC risk for AA women. Although this is a large prospective study of ~60 000 women, the inferences were limited by the small number of AA ovarian cancer cases ($n = 41$). Other studies including AA women had relatively small samples of AA women with inadequate power to assess race-specific associations (Schildkraut *et al*, 2006; Wu *et al*, 2009). To overcome these challenges, the present analysis utilised the largest study of EOC in AA women, to date, to examine EOC risk associated with analgesic medication use exclusively among AA women.

MATERIALS AND METHODS

Study population. The African American Cancer Epidemiology Study (AACES) is a population-based case–control study examining risk factors for EOC exclusively among AA women. The AACES is a collaborative effort between 11 sites, including Alabama, Georgia, Illinois, Louisiana, Michigan, New Jersey, North Carolina, Ohio, South Carolina, Tennessee, and Texas. A detailed description of methods for AACES has been published elsewhere (Schildkraut *et al*, 2014). Briefly, cases were identified through rapid case ascertainment at SEER and state cancer registries, gynaecologic oncology departments, or hospitals. Eligibility criteria for the cases included: self-identification of AA race, aged 20–79 years, and newly diagnosed with invasive EOC after December 2010. The AA controls were identified through random digit dialing, and were frequency matched to cases by 5-year age category and state of residence. Women were excluded if previously diagnosed with EOC or if they had a bilateral oophorectomy. The AACES participants completed a telephone interview, including questions on demographic characteristics, reproductive history, oral contraceptive use, hormone therapy, family history of cancers, medication use, and a variety of lifestyle characteristics (e.g., smoking, physical activity). A short form of the questionnaire could also be completed in an effort to increase participation for women who would have otherwise refused. The study protocol was approved by the Institutional Review Board at each site, and informed consent was obtained for all participants.

As of August 2015, AACES has enrolled a total of 593 cases and 750 controls ($N = 1343$). Of these, 71 women completed the short questionnaire, 52 cases and 19 controls. As the short form of the questionnaire did not inquire about analgesic medication use, the data set was restricted to include only those women completing the long form of the questionnaire ($N = 1272$; 541 cases and 731 controls).

Analgesic drug use. In the questionnaire, participants were asked to recall any medications for pain or inflammation that were taken regularly, defined as at least once a week or at least 5 days out of the month, at any point in their lifetime. Examples of analgesic drugs and indications of use were provided to aid in recollection. Women who responded affirmatively to ever using pain or inflammation medications were then asked the name of the drug (including dosing information, if available), the reason for using the drug, how many days per month taken, age of first and last use, and the duration of use in months or years. This series of questions was repeated if the participant reported using more than one drug in her lifetime, with no participant reporting use of more than 10 analgesic drugs. The names of each drug were reviewed and categorised into the following groups: aspirin, non-aspirin NSAIDs, and acetaminophen. Some medications contained a combination of these analgesic types (e.g., aspirin and acetaminophen) and women who reported taking them were categorised as having used both types of analgesics. Any reported medication that did not fit into one of these categories (e.g., muscle relaxants, opioids, anti-epileptics) was considered as nonuse. To successfully model a time period of analgesic use that was not influenced by potential symptoms of an undiagnosed EOC, women who reported initiation of analgesic drug use within the year before the reference date (cases: diagnosis of EOC; controls: time of interview) were categorised as nonusers. In addition, a women who reported a duration of use of <6 months or any case who reported initiation of analgesic drug use after her EOC diagnosis was categorised as a nonuser.

Statistical analyses. Multivariate unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the relationship between use of analgesic medications and risk of EOC. Each medication type was examined in a separate model with women who never used any analgesic medication serving as the referent group. In addition, the association of any type of NSAID (aspirin and non-aspirin NSAIDs, but not acetaminophen) with EOC risk was also examined. Use of aspirin, non-aspirin NSAIDs, and acetaminophen was further examined by frequency of use (<30 times per month, daily), duration of use (<5 years, $\geqslant 5$ years), combined frequency and duration of use (<30 times per month for <5 years, daily for <5 years, <30 times per month for $\geqslant 5$ years, daily for $\geqslant 5$ years), and indication of use (arthritis, menstrual cramps, injury/pain, headache, and heart disease). Because of insufficient data on dose for non-aspirin NSAIDs and acetaminophen, the

effect of dose on EOC risk was assessed for aspirin only (low dose: <100 mg; high dose: $\geqslant 100$ mg).

The following *a priori* confounders were adjusted for in all models: age (age at diagnosis for cases and age at the time of interview for controls); study site (Alabama, Georgia and Tennessee combined (because of small sample sizes and geographic similarities), Illinois and Michigan combined (because of small sample sizes and geographic similarities), Louisiana, New Jersey, North Carolina, Ohio, South Carolina, and Texas); education (high school graduate or less, some post-high school training, and college or graduate degree); income ($<\$25\,000$, \$25 000–\$49 999, \$50 000–\$74 999, and $\geqslant\$75\,000$); parity (nulliparous, 1, 2, 3, or more live births); family history of a first-degree relative with breast or ovarian cancer (yes, no); tubal ligation (yes, no); body mass index ($<25\,kg\,m^{-2}$: underweight and normal weight; 25–29.9 $kg\,m^{-2}$: overweight; $\geqslant 30\,kg\,m^{-2}$: obese); oral contraceptive use (ever, never); menopausal status (pre-, peri-, and post-menopause); endometriosis (yes, no); pelvic inflammatory disease (yes, no); mild physical activity (yes, no); and moderate or strenuous physical activity (yes, no). Several comorbid conditions (e.g., heart disease, osteoporosis, and arthritis) were also evaluated as potential confounders, but no appreciable change in the effect estimates was observed and these conditions were not included in the final models. As women commonly reported use of more than one type of analgesic (e.g., of the women reporting acetaminophen use, 40% also reported using non-aspirin NSAIDs), use of (frequency of, duration of, indication of) the other analgesic types were simultaneously adjusted for in regression models. Therefore, the estimated associations in the present study reflect the independent effect of that type of analgesic without confounding by use of other analgesic types.

Finally, potential effect modification by two pro-inflammatory factors, BMI and smoking, was evaluated to determine whether analgesic medication use may be particularly beneficial for specific subgroups with higher inflammation. All analyses were conducted using SAS, Version 9.3 (SAS, Cary, NC, USA).

RESULTS

A total of 1272 subjects, 541 cases and 731 controls, were included in the analysis. Table 1 describes demographic, reproductive, and lifestyle characteristics of cases and controls. Compared with controls, cases were more likely to be older (because of collapsing of 5-year age categories that were used for frequency matching), to have a high school education or less, to be nulliparous, to have a family history of a first-degree relative with breast or ovarian cancer, to have had endometriosis, and to have had pelvic inflammatory disease, and cases were less likely to have had tubal ligation, to use oral contraceptives, and to engage in mild intensity physical activity. The majority of cases were diagnosed with serous EOC (72.8%). Overall, 467 women reported use of any analgesic medication (36.7%). Of those, non-aspirin NSAIDs were the most commonly reported analgesic medication (62.7%), followed by aspirin and acetaminophen, 35.1% and 27.4% respectively (percentages do not equal 100% because of women taking more than one type of analgesic drug).

The associations between analgesic medication use and EOC risk are shown in Table 2. In comparison with never users of any analgesic medications, women who used any type of NSAIDs, including aspirin and non-aspirin NSAIDs but not acetaminophen, had a 27% lower risk of EOC (OR = 0.73, 95% CI = 0.54–0.98). No association with EOC risk was observed for women who used a combination of all analgesic drug types (OR = 1.03, 95% CI = 0.58–1.84). When evaluating use of each analgesic medication in separate models, a statistically significant 44% lower EOC risk was observed for women who reported use of aspirin (OR = 0.56, 95% CI = 0.35–0.92), and a 26% lower EOC risk (OR = 0.74, 95% CI = 0.52–1.05) was observed for non-aspirin NSAID use. Acetaminophen was inversely associated with risk of EOC, although not statistically significant (OR = 0.89, 95% CI = 0.49–1.62).

Table 3 shows the associations for frequency and duration of use for each analgesic drug and risk of EOC. Irrespective of frequency and duration of aspirin use, inverse associations with risk of EOC were observed. Statistically significant lower risks of EOC were observed for daily aspirin use (OR = 0.56, 95% CI = 0.34–0.94) and aspirin use for a duration of <5 years (OR = 0.52, 95% CI = 0.28–0.98). A significant inverse association with risk of EOC was observed for AA women who occasionally used non-aspirin NSAIDs (<30 days per month), OR = 0.54 (95% CI = 0.35–0.83). An inverse association was observed for women who used non-aspirin NSAIDs for a duration of $\geqslant 5$ years, although not statistically significant (OR = 0.71, 95% CI = 0.47–1.07). In combined analyses of frequency and duration of use, inverse associations with EOC risk, although not statistically significant, were observed for all categories of aspirin use. However, a statistically significant lower risk of EOC was present among women who used non-aspirin NSAIDs for <30 days per month for a duration of $\geqslant 5$ years (OR = 0.47, 95% CI = 0.28–0.79). Although inversely associated, no statistically significant associations were observed for frequency or duration of acetaminophen use.

The most common indication of aspirin use was heart disease prevention (75.3%), whereas arthritis (45.5%) was the most common reason for non-aspirin NSAID use. Significant inverse associations were observed for the most prevalent indication of use for aspirin and non-aspirin NSAIDs, where a 50% lower EOC risk was observed for women using aspirin to prevent heart disease (OR = 0.50, 95% CI = 0.29–0.85) and a 48% lower EOC risk for women using non-aspirin NSAIDs for arthritis (OR = 0.52, 95% CI = 0.31–0.88) (data not shown).

The influence of dose for analgesic medications on EOC risk was also examined; however, sufficient data on dose were only present for aspirin use. Of the aspirin users with dosing information ($n = 127$), 77.2% reported low-dose aspirin use. Low-dose aspirin users had a more pronounced, statistically significant inverse association with EOC risk, OR = 0.54 (95% CI = 0.31–0.97), in comparison with high-dose aspirin users, OR = 0.78 (95% CI = 0.30–2.06) (data not shown).

Presence of effect modification by BMI and smoking on the relationship between analgesic medications and EOC risk was not observed, $P<0.05$ (data not shown).

DISCUSSION

This is the first study to examine the association between analgesic drug use and EOC risk in a large population of AA women. A statistically significant 44% lower EOC risk and a borderline significant 26% lower EOC risk was observed among aspirin and non-aspirin NSAID users, respectively. Although inversely related, no significant associations for acetaminophen use and EOC risk were observed in this population. The inverse association between analgesic use and EOC was consistent with previous findings from several studies (Schildkraut *et al*, 2006; Wernli *et al*, 2008; Pinheiro *et al*, 2009), and especially parallel the findings in the recent pooled analysis (Trabert *et al*, 2014). As with the pooled analysis, a similar inverse association for aspirin use and EOC risk, overall, was observed and especially for low-dose aspirin use, daily aspirin use, and shorter duration of aspirin use (<5 years). The present study also observed a decreased EOC risk for non-aspirin NSAID use

Table 1. Characteristics[a] of cases and controls, AACES (*N* = 1272)

	Cases (*n* = 541)	Controls (*n* = 731)	
	N (%)	*N* (%)	*P*-value
Age, years[b]			
<50	124 (23.1)	198 (27.1)	0.02
50–59	188 (35.1)	272 (37.2)	
60–69	143 (26.7)	189 (25.8)	
70+	81 (15.1)	72 (9.9)	
Education			
⩽High school	242 (44.7)	271 (37.1)	0.02
Some post high school training	139 (25.7)	207 (28.3)	
College or graduate degree	160 (29.6)	253 (34.6)	
Income			
<$25 000	254 (47.9)	320 (44.3)	0.18
$25 000–$49 999	130 (24.5)	162 (22.4)	
$50 000–$74 999	76 (14.4)	121 (16.7)	
$75 000+	70 (13.2)	120 (16.6)	
Parity (number of live births)			
Nulliparous	105 (19.4)	93 (12.7)	0.01
1	101 (18.7)	137 (18.7)	
2	123 (22.7)	194 (26.6)	
3+	212 (39.2)	307 (42.0)	
Family history of breast or ovarian cancer			
No	383 (72.5)	577 (81.6)	0.0002
Yes	145 (27.5)	130 (18.4)	
Tubal ligation			
No	351 (64.9)	431 (59.0)	0.03
Yes	190 (35.1)	300 (41.0)	
BMI (kg m^{-2})			
<25 (underweight and normal weight)	79 (14.7)	136 (18.6)	0.16
25–29.9 (overweight)	139 (25.8)	189 (25.9)	
30+ (obese)	320 (59.5)	405 (55.5)	
Oral contraceptive use			
Never	163 (30.1)	151 (20.7)	0.0001
Ever	378 (69.9)	580 (79.3)	
Menopausal status			
Pre-menopausal	84 (15.6)	134 (18.4)	0.29
Peri-menopausal	73 (13.5)	84 (11.5)	
Post-menopausal	382 (70.9)	511 (70.1)	
Endometriosis			
No	476 (88.6)	696 (95.2)	0.00001
Yes	61 (11.4)	35 (4.8)	
Pelvic inflammatory disease			
No	491 (91.4)	692 (94.9)	0.01
Yes	46 (8.6)	37 (5.1)	
Mild intensity physical activity			
No	389 (72.3)	462 (63.2)	0.0007
Yes	149 (27.7)	269 (36.8)	
Moderate or strenuous intensity physical activity			
No	301 (55.7)	426 (58.3)	0.37
Yes	239 (44.3)	305 (41.7)	
Histologic subtype			
Serous	367 (72.8)		
Mucinous	24 (4.7)		
Endometrioid	70 (13.9)		
Clear cell	12 (2.4)		
Mixed	14 (2.8)		
Other	17 (3.4)		

Abbreviations: AACES = African American Cancer Epidemiology Study; BMI = body mass index.
[a]Missing data on age for 5 women, income for 19 women, family history of a first-degree relative with breast or ovarian cancer for 37 women, BMI for 4 women, menopausal status for 4 women, endometriosis for 4 women, pelvic inflammatory disease for 6 women, mild intensity physical activity for 3 women, and moderate or strenuous intensity physical activity for 1 woman, and 37 cases for histologic subtype.
[b]Age at diagnosis for cases and age at the time of interview for controls.

overall and specifically for occasional monthly use (<30 days per month) for ⩾5 years. The previously published pooled analysis only observed a significant inverse association for high-dose non-aspirin NSAID use (Trabert *et al*, 2014); however, the non-aspirin NSAID use results of the present study were similar to many published studies (Fairfield *et al*, 2002; Schildkraut *et al*, 2006; Merritt *et al*, 2008; Wernli *et al*, 2008; Pinheiro *et al*, 2009).

Interestingly, the inverse relationship between analgesic use and EOC risk observed in the present study was more pronounced than those observed in predominately white populations. In the only other study to conduct race-specific analyses for analgesic use and EOC risk, AA women had the strongest inverse association between aspirin and non-aspirin NSAID use and EOC risk, although not significant (Setiawan *et al*, 2012). Similar differences in the magnitude of effect by race have been reported for breast cancer, where use of analgesic medications was associated with a stronger inverse relationship for risk of breast cancer among AA women compared with all other races (Gill *et al*, 2007). As AA women have higher levels of inflammation, in general, compared with white women (Albert *et al*, 2004; Khera *et al*, 2005; Paalani *et al*, 2011), the potential benefit of taking anti-inflammatory drugs may be greater among AA women. The observed racial differences may also be because of a variety of factors that could vary by race, including genetic variants (e.g., prevalence of polymorphisms affecting cyclooxygenase activity), cultural attitudes toward analgesic use, or patterns of analgesic use. Further research needs to be conducted among racially diverse populations to confirm the observed racial differences in effect.

Although our findings suggest a protective effect for aspirin taken daily, at low doses, and for heart disease prevention, these three characteristics of use are highly correlated. A daily, low dose of aspirin is typically recommended for women at high risk of a cardiovascular event. In the WaTCH study, of the women who took aspirin for heart disease prevention, >85% also reported low-dose aspirin use and a daily frequency of use. As the overwhelming majority of women taking aspirin for heart disease are the same women using aspirin daily and at low doses, it is difficult to determine whether indication, frequency, dose, or a combination of these characteristics of use is contributing to the reduction in risk. In addition, the observed association between aspirin use for heart disease prevention and EOC risk in the present study may be explained by the effects of other cardiovascular drugs (e.g., β-blockers, statins, angiotensin-converting enzyme inhibitors) taken concurrently with analgesic medications. To explore this possibility, the cardiovascular medication use of women taking aspirin for heart disease prevention ($n = 122$) was examined; although a slightly higher prevalence of these cardiovascular medications was taken by the controls, a statistically significant difference in drug use was not observed (data not shown).

Although ovarian cancer is associated with a long latency period, our findings suggest that shorter durations of aspirin use (<5 years) confer a stronger protective effect. Previous literature evaluating this relationship is inconsistent; some studies (Akhmedkhanov *et al*, 2001; Wernli *et al*, 2008), including the large pooled analysis (Trabert *et al*, 2014), observed a stronger inverse association for shorter durations of aspirin use, whereas other studies reported a stronger protection for longer durations of use (Rosenberg *et al*, 2000; Lacey *et al*, 2004; Schildkraut *et al*, 2006). Although unclear, we speculate that the findings for shorter durations of use could be because of a few factors. A recall bias may be present, resulting in misclassification of duration. Women may have had difficulty accurately recalling their duration of use if it occurred for a longer period of time. In addition, as analgesic medications are routinely used for a variety of indications, women may not keep track of each use, leading to an underestimation of duration. The majority of women reporting a short duration of aspirin use began taking these medications within 2–5 years of their diagnosis (cases) or the time of interview (controls). It is

possible that using aspirin during this time period may be more beneficial in protecting against ovarian cancer progression. In fact, cyclooxygenase inhibitors (i.e., aspirin) have been shown to decrease cell growth, increase apoptosis, and block angiogenesis (Dubois *et al*, 1998). Experimental studies are needed to explore a mechanistic explanation as to why a shorter duration of aspirin use may provide a greater protection against ovarian cancer.

The present study has several strengths. First, data from the largest study of EOC among AA women were utilised, resulting in a relatively large sample size to examine race-specific associations for analgesic use. Another strength is the detailed exposure assessment of analgesic use, allowing for effect estimates by frequency, duration, and indication of use. Despite these strengths, there were several limitations present in this study. Data on analgesic drug use were ascertained through self-report and may be subject to potential biases. In particular, if participants used several different types of these medications during their lifetime, it may be difficult to accurately report their use of each, especially with regard to the duration, frequency, and indication of use. However, the associations observed in the present study are consistent with those from the large pooled analysis of the Ovarian Cancer Association Consortium (Trabert *et al*, 2014) and other cohort studies (Setiawan *et al*, 2012), although not significant. In addition, women with cardiovascular disease may be more acutely aware of their aspirin use; however, the prevalence of heart disease is similar among cases and controls (~11%) and any resulting misclassification would bias the effect estimates towards the null. The lack of information on dose for non-aspirin NSAIDs and acetaminophen use was another limitation in this study. Because of the case–control study design, a potential selection bias may be present (i.e., women using analgesic medications may be more healthy and more likely to participate in the study). To evaluate the potential for a selection bias, the prevalence of analgesic use among AACES controls was compared with the available estimates for the general US population (Zhou *et al*, 2014). Although a direct comparison group for the demographic of the AACES study was unavailable, the prevalence of aspirin use among Black adults, aged ⩾18 years, was similar to AACES controls, and although possible, a selection bias is unlikely. Finally, although AACES is the largest study of EOC in AA, the sample of AA women within certain categories of exposure (e.g., indication of aspirin use, combined frequency, and duration of use) was small, limiting the power and precision of the effect estimates.

Table 2. Estimated ORs and 95% CIs for the association of analgesic medication use and ovarian cancer risk

Analgesic medication use			
	No. of cases (*n* = 541)	**No. of controls (*n* = 731)**	**Adjusted OR[a] (95% CI)**
Never users	362	467	1.00 (Referent)
Any NSAID[b] only	125	197	0.73 (0.54–0.98)
Acetaminophen only	23	33	0.98 (0.53–1.81)
Both NSAIDs and acetaminophen	31	34	1.03 (0.58–1.84)
Aspirin use			
	No. of cases (*n* = 432)	**No. of controls (*n* = 560)**	**Adjusted OR[a,c] (95% CI)**
Never users	362	467	1.00 (Referent)
Ever use	70	93	0.56 (0.35–0.92)
Non-aspirin NSAID use			
	No. of cases (*n* = 474)	**No. of controls (*n* = 628)**	**Adjusted OR[a,c] (95% CI)**
Never users	362	467	1.00 (Referent)
Ever use	112	161	0.74 (0.52–1.05)
Acetaminophen use			
	No. of cases (*n* = 416)	**No. of controls (*n* = 534)**	**Adjusted OR[a,c] (95% CI)**
Never users	362	467	1.00 (Referent)
Ever use	54	67	0.89 (0.49–1.62)

Abbreviations: CI = confidence interval; NSAID = nonsteroidal anti-inflammatory drug; OR = odds ratio.
[a]Adjusted for age, study site, education, income, parity, family history of first-degree relative with breast or ovarian cancer, tubal ligation, body mass index (BMI), oral contraceptive use, menopausal status, endometriosis, pelvic inflammatory disease, and physical activity.
[b]Any NSAID use includes aspirin and non-aspirin NSAIDs, but not acetaminophen.
[c]Also simultaneously adjusted for use of other analgesics.

Table 3. Estimated ORs and 95% CIs for the association of frequency and duration of aspirin, non-aspirin NSAIDs, and acetaminophen use with ovarian cancer risk

	Aspirin			**Non-aspirin NSAID**			**Acetaminophen**		
	No. of cases (*n* = 432)	**No. of controls (*n* = 560)**	**Adjusted OR[a] (95% CI)**	**No. of cases (*n* = 474)**	**No. of controls (*n* = 628)**	**Adjusted OR[a] (95% CI)**	**No. of cases (*n* = 416)**	**No. of controls (*n* = 534)**	**Adjusted OR[a] (95% CI)**
Never users	362	467	1.00 (Referent)	362	467	1.00 (Referent)	362	467	1.00 (Referent)
Frequency of use									
<30 Days/month	15	27	0.51 (0.21–1.25)	58	108	0.54 (0.35–0.83)	35	48	0.94 (0.49–1.81)
Daily	55	66	0.56 (0.34–0.94)	54	53	1.15 (0.70–1.91)	19	19	0.79 (0.33–1.92)
Duration of use									
<5 Years	27	41	0.52 (0.28–0.98)	33	51	0.81 (0.48–1.37)	15	25	0.97 (0.42–2.22)
⩾5 Years	43	52	0.60 (0.32–1.11)	79	110	0.71 (0.47–1.07)	39	42	0.87 (0.44–1.74)
Frequency, duration of use									
<30 Days/month for <5 years	7	13	0.43 (0.14–1.34)	20	33	0.67 (0.35–1.27)	8	18	0.94 (0.34–2.63)
Daily for <5 years	20	28	0.56 (0.27–1.11)	13	18	1.13 (0.48–2.66)	7	7	1.12 (0.32–3.96)
<30 Days/month for ⩾5 years	8	14	0.61 (0.17–2.22)	38	75	0.47 (0.28–0.79)	27	30	1.00 (0.45–2.21)
Daily for ⩾5 years	35	38	0.56 (0.29–1.08)	41	35	1.17 (0.65–2.09)	12	12	0.58 (0.19–1.79)

Abbreviations: CI = confidence interval; NSAID = nonsteroidal anti-inflammatory drug; OR = odds ratio.
[a]Adjusted for age, study site, education, income, parity, family history of first-degree relative with breast or ovarian cancer, tubal ligation, body mass index (BMI), oral contraceptive use, menopausal status, endometriosis, pelvic inflammatory disease, physical activity, and simultaneous adjustment of use of other analgesics (frequency, duration).

In conclusion, this study supports previous evidence that any NSAID use, but not acetaminophen, is inversely associated with EOC risk. The findings of the present study raise the intriguing possibility that the inverse association may be stronger in AA women compared with white women. This possibility emphasises the value of studying this question among racially diverse populations. Future research, specifically in large cohort studies, is needed in order to fully elucidate the impact of analgesic drug use on EOC risk in AA women, as well as other underrepresented racial groups.

ACKNOWLEDGEMENTS

This study was supported by the National Cancer Institute (Grant CA142081). Additional support was provided by the Metropolitan Detroit Cancer Surveillance System with funding from the National Cancer Institute, National Institute of Health, the Department of Health and Human Services (Contract HHSN261201000028C), and the Epidemiology Research Core, supported in part by the National Cancer Institute Center (Grant P30 CA22453) to the Karmanos Cancer Institute, Wayne State University School of Medicine). We are grateful to the AACES interviewers, Christine Bard, LaTonda Briggs, Whitney Franz (North Carolina), and Robin Gold (Detroit). We also acknowledge the individuals responsible for facilitating case ascertainment across all AACES sites.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Exhibit 115

original reports

Modification of the Association Between Frequent Aspirin Use and Ovarian Cancer Risk: A Meta-Analysis Using Individual-Level Data From Two Ovarian Cancer Consortia

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abstract

PURPOSE Frequent aspirin use has been associated with reduced ovarian cancer risk, but no study has comprehensively assessed for effect modification. We leveraged harmonized, individual-level data from 17 studies to examine the association between frequent aspirin use and ovarian cancer risk, overall and across subgroups of women with other ovarian cancer risk factors.

METHODS Nine cohort studies from the Ovarian Cancer Cohort Consortium (n = 2,600 cases) and eight case-control studies from the Ovarian Cancer Association Consortium (n = 5,726 cases) were included. We used Cox regression and logistic regression to assess study-specific associations between frequent aspirin use (≥ 6 days/week) and ovarian cancer risk and combined study-specific estimates using random-effects meta-analysis. We conducted analyses within subgroups defined by individual ovarian cancer risk factors (endometriosis, obesity, family history of breast/ovarian cancer, nulliparity, oral contraceptive use, and tubal ligation) and by number of risk factors (0, 1, and ≥ 2).

RESULTS Overall, frequent aspirin use was associated with a 13% reduction in ovarian cancer risk (95% CI, 6 to 20), with no significant heterogeneity by study design (P = .48) or histotype (P = .60). Although no association was observed among women with endometriosis, consistent risk reductions were observed among all other subgroups defined by ovarian cancer risk factors (relative risks ranging from 0.79 to 0.93, all P-heterogeneity > .05), including women with ≥ 2 risk factors (relative risk, 0.81; 95% CI, 0.73 to 0.90).

CONCLUSION This study, the largest to-date on aspirin use and ovarian cancer, provides evidence that frequent aspirin use is associated with lower ovarian cancer risk regardless of the presence of most other ovarian cancer risk factors. Risk reductions were also observed among women with multiple risk factors, providing proof of principle that chemoprevention programs with frequent aspirin use could target higher-risk subgroups.

J Clin Oncol 00. Published by American Society of Clinical Oncology

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on June 17, 2022 and published at ascopubs.org/journal/jco on July 22, 2022: DOI https://doi.org/10.1200/JCO.21.01900

INTRODUCTION

Ovarian cancer is the most fatal gynecologic cancer, largely because of nonspecific symptom presentation and lack of early detection strategies.[1] Chemoprevention holds promise but remains an understudied paradigm to reduce ovarian cancer burden.[2] Chronic inflammation likely plays a key role in ovarian carcinogenesis,[3] as factors associated with epithelial disruption from ovulation,[4,5] inflammation-related exposures such as endometriosis and pelvic inflammatory disease,[6,7] and circulating biomarkers of inflammation[8,9] are associated with ovarian cancer risk. Anti-inflammatory medications such as aspirin may lower risk of ovarian cancer development via inhibition of the cyclooxygenase enzymes, leading to decreased synthesis of inflammatory mediators, or via cyclooxygenase-independent pathways including inhibition of Wnt/β-catenin and NF-κβ.[10]

A growing body of evidence supports a role of aspirin in reducing ovarian cancer risk. Pooled secondary analyses of randomized controlled trials of aspirin for cardiovascular disease prevention

CONTEXT

Key Objective

To determine whether the association between frequent aspirin use and ovarian cancer risk is modified by established ovarian cancer risk factors (endometriosis, obesity, family history of breast/ovarian cancer, parity, oral contraceptive use, and tubal ligation).

Knowledge Generated

In combined analyses of individual participant data from 17 study populations, frequent aspirin use was associated with a 13% reduction in ovarian cancer risk overall. Consistent risk reductions were observed across most subgroups of women with other ovarian cancer risk factors, with the exception of endometriosis. Among women with two or more risk factors, frequent aspirin use was associated with a 19% reduction in ovarian cancer risk.

Relevance

This study confirms the association of frequent aspirin use with decreased risk of ovarian cancer. The use of aspirin for ovarian cancer chemoprevention may best be targeted to higher-risk women with two or more ovarian cancer risk factors, to maximize the population-level benefit/risk ratio.

have noted a decreased risk of female reproductive cancers with ≥ 3 years of aspirin use, although too few ovarian cancer cases were diagnosed in these trial populations to draw inferences for ovarian cancer specifically.[11] In the observational setting, individual study results have been mixed,[12-23] but meta-analyses[24] and pooled analyses of cohort[25] and case-control[26] studies have found that aspirin may reduce ovarian cancer risk by 10%-20%, particularly when used frequently (ie, daily or almost daily).

However, although aspirin use appears to be one of the few modifiable protective factors for ovarian cancer, population-wide chemoprevention programs are generally considered infeasible because of the low incidence of ovarian cancer and the known risk of bleeding conferred by frequent aspirin use.[27] Instead, such programs will likely need to focus on subgroups of women at elevated risk of ovarian cancer.[28] Established factors that increase ovarian cancer risk include a family history of breast or ovarian cancer, endometriosis, and obesity, whereas factors that decrease risk include parity, oral contraceptive use, and tubal ligation. Whether frequent aspirin use reduces risk of ovarian cancer among subgroups of women defined by these risk factors is unknown, and extremely large, well-powered studies are needed.

In this study, we leveraged harmonized, individual-level data from two ovarian cancer consortia that previously reported on frequent aspirin and ovarian cancer risk[25,26] to comprehensively assess this association across key subgroups of interest. By meta-analyzing results from these 17 studies, we aimed to test for the consistency of the association across study design and personal characteristics and provide the most precise estimates of the aspirin-ovarian cancer association to date.

METHODS

Study Populations

We analyzed individual-level data from prospective cohort studies from the Ovarian Cancer Cohort Consortium (OC3)[29] and population-based case-control studies from the Ovarian Cancer Association Consortium (OCAC). Studies were included if they collected information on frequency of aspirin use; this resulted in the inclusion of nine cohort and eight case-control studies, a subset of the studies included in previous aspirin analyses from these consortia.[25,26] The cohort studies (NIH-AARP Diet and Health Study,[16,30] Cancer Prevention Study II Nutrition Cohort,[31,32] California Teachers Study,[33] Iowa Women's Health Study,[19] Nurses' Health Study,[18] Nurses' Health Study II,[18] Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial,[34] Sister Study,[35] and Vitamins and Lifestyle Cohort[36,37]) were all US-based cohorts; our analysis included women from these cohorts with at least one intact ovary, no history of cancer at baseline, and nonmissing age and frequency of aspirin use. The case-control studies (Australian Ovarian Cancer Study,[22] Diseases of the Ovary and their Evaluation Study,[23,38] Hawaii Ovarian Cancer Study,[39,40] Hormones and Ovarian Cancer Prediction Study,[41] North Carolina Ovarian Cancer Study,[42,43] University of California, Irvine Ovarian Cancer Study,[44] United Kingdom Ovarian Cancer Population Study,[45] University of Southern California, and Study of Lifestyle and Women's Health[46]) were from the United States, United Kingdom, and Australia.

All participating studies obtained institutional review board approval at their respective institutions. Participants provided written informed consent or implicit consent through return of study questionnaires. The coordinating centers for OC3 (Brigham and Women's Hospital, Harvard T. H. Chan School of Public Health) and OCAC (Duke University)

received institutional review board approval from their institutions and participating registries as required for data acquisition, pooling, and harmonization.

Study Variables

Given previous findings that frequent aspirin use was most strongly associated with ovarian cancer risk,[25,26] our primary exposure was frequent aspirin use, which was self-reported in all included studies (Appendix Tables A1 and A2, online only). Frequent aspirin use was harmonized across the study populations to indicate aspirin use for ≥ 6 days/week or ≥ 28 days/month and for a duration of ≥ 6 months. Frequent aspirin use was defined irrespective of dose, as few studies collected data on aspirin dose. Women who reported less frequent or no aspirin use were combined to form the reference group. Other covariates were centrally harmonized at the coordinating centers of OC3 and OCAC.[6,29,47-49] For the cohort studies, aspirin use and other covariates were assessed at enrollment or at a subsequent questionnaire cycle, which then became the baseline for this analysis. For the case-control studies, covariates were ascertained at enrollment.

Our primary outcome was invasive epithelial ovarian, fallopian tube, or peritoneal cancer. In the cohort studies, cases were identified through linkage to cancer registries or medical chart review.[29] Nonepithelial tumors and tumors of low malignant potential/borderline were excluded. Case ascertainment for the case-control studies included linkage to cancer registries or hospital registries, surgical treatment centers, gynecologic oncology centers, physician offices, and/or pathology databases.[47] We also examined associations for the most common ovarian cancer histotypes, including high-grade serous, mucinous, endometrioid, clear cell, and other epithelial tumors. Very few low-grade serous tumors were observed in these study populations; these tumors were consequently excluded from histotype-specific analyses.

Statistical Analysis

For each cohort study, hazard ratios (HRs) and 95% CIs comparing frequent aspirin use to nonfrequent use were calculated using Cox proportional hazards regression. Women entered the analysis at age at study entry and contributed person-time until first diagnosis of ovarian cancer, death, or end of follow-up. Models were adjusted for baseline age, number of full-term births (none, one, two, three, or ≥ four), duration of oral contraception use (never, ≤ 1, > 1-5, > 5-10, or > 10 years), duration of menopausal hormone therapy use (premenopausal, never, ≤ 5, > 5-10, or > 10 years), and body mass index (BMI, < 20, 20 to < 25, 25 to < 30, 30 to < 35, or ≥ 35 kg/m^2). For each case-control study, odds ratios (ORs) and 95% CIs were calculated using logistic regression, adjusting for the same covariates. Study-specific HRs and ORs were calculated overall as well as for subgroups defined by age at baseline (cohort studies) or diagnosis/index date (case-control studies; < 50, 50-59, 60-69, or ≥ 70 years), history of endometriosis (yes or no), obesity (BMI ≥ 30 or < 30 kg/m^2), parity (parous or nulliparous), family history of breast or ovarian cancer (yes or no), duration of oral contraceptive use (never, < 5, or ≥ 5 years), tubal ligation (yes or no), and nonaspirin nonsteroidal anti-inflammatory drug (NSAID) use (yes or no). Study-specific effect estimates, overall and for each subgroup, were combined using random effects meta-analysis to generate summary relative risks (RRs).

We also calculated RRs within subgroups defined by an ovarian cancer risk score (range, 0-6, categorized as 0, 1, and ≥ 2), with each ovarian cancer risk factor (endometriosis, obesity, nulliparity, family history of breast or ovarian cancer, no oral contraceptive use, and no tubal ligation) contributing one point to this score. Before using this score, we confirmed that the risk score was positively associated with ovarian cancer risk (RR for a score of 1 *v* 0: 1.20, 95% CI, 1.10 to 1.30; RR for a score of ≥ 2 *v* 0: 1.78, 95% CI, 1.64 to 1.94). Risk score analyses were adjusted for age and duration of menopausal hormone therapy use.

To examine associations by ovarian cancer histotype, we conducted competing risks Cox regression using an augmented data approach with the pooled cohort data,[50] and polytomous logistic regression with the pooled case-control data,[51,52] adjusting for study and the same covariates as above. We conducted pooled instead of study-specific analyses because of the smaller number of events by histotype. The results from the cohort and case-control analyses were combined using random effects meta-analysis.

We examined heterogeneity in effect estimates by study, study design, subgroup, and histotype using Cochran's Q tests.[53] The number needed to treat (NNT) to prevent one ovarian cancer was calculated using the observed 10-year absolute risk of ovarian cancer among nonaspirin users in the cohort studies and the combined cohort and case-control summary RRs.[54] All statistical tests were two-sided, and *P* values < .05 were considered statistically significant. Study-specific and pooled analyses were conducted in SAS 9.4, meta-analyses were conducted using the meta command in Stata 16, and figures were generated in R 4.0.2.

RESULTS

In the nine cohort studies, there were 491,651 women at risk. The mean age at baseline ranged from 46.0 to 68.2 years, mean follow-up ranged from 4.6 to 14.3 years, and the prevalence of frequent aspirin use ranged from 9.8% to 38%. During follow-up, 2,600 women were diagnosed with incident ovarian cancer (56% high-grade serous, 2% low-grade serous, 9% endometrioid, 5% clear cell, 4% mucinous, and 23% other/unknown epithelial). Across the eight case-control studies, there were

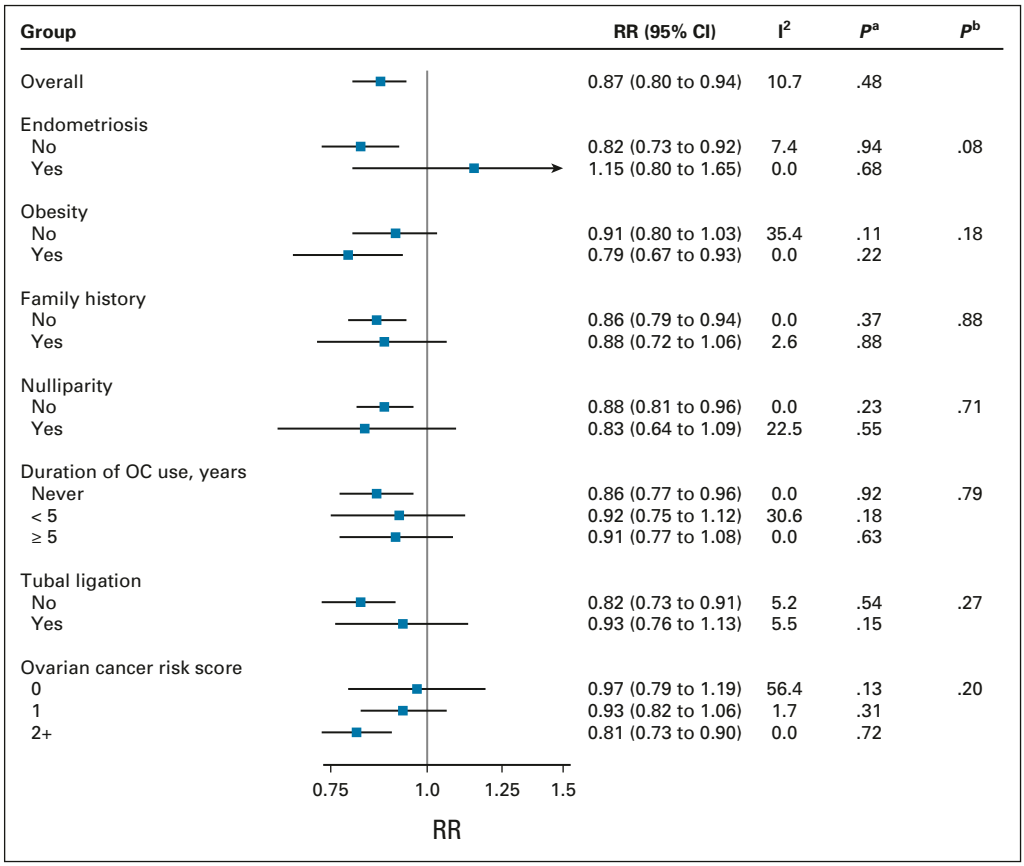

FIG 1. Summary RRs for the association between frequent aspirin use and ovarian cancer risk in OC3 and OCAC, overall and by key subgroups of interest. Number of studies included in subgroup-specific meta-analyses: endometriosis (n = 11), obesity (n = 16), family history of breast/ovarian cancer (n = 15 for no/ n = 16 for yes), parity (n = 17), duration of OC use (n = 16), tubal ligation (n = 14), and risk score (n = 17). Models were adjusted for age, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use, and BMI. Models stratified by risk score were adjusted for age and duration of menopausal hormone therapy use. Participants with missing data on these covariates (< 10% for all covariates except duration of menopausal hormone therapy use) were retained in the models using missing indicators. We also conducted a complete case analysis and the results were unchanged. [a]*P* value for heterogeneity by study design. [b]*P* value for heterogeneity by subgroup. BMI, body mass index; OC, oral contraceptive; OC3, Ovarian Cancer Cohort Consortium; OCAC, Ovarian Cancer Association Consortium; RR, relative risk.

5,726 cases (54% high-grade serous, 4% low-grade serous, 15% endometrioid, 9% clear cell, 5% mucinous, and 13% other/unknown epithelial) and 8,027 controls. The median age of the cases ranged from 56.2 to 60.7 years, and the prevalence of frequent aspirin use ranged from 5.6% to 29.8%. Additional characteristics of the study populations are described in Appendix Tables A1 and A2.

Overall, frequent aspirin use was associated with a 10% reduction in ovarian cancer risk in the cohort studies (HR, 0.90; 95% CI, 0.81 to 1.01) and a 16% reduced risk in the case-control studies (OR, 0.84; 95%, CI, 0.72 to 0.98, Appendix Fig A1, online only). Meta-analyzing the cohort and case-control studies yielded an overall summary RR of 0.87 (95% CI, 0.80 to 0.94), with no difference between the cohort and case-control study results (*P*-heterogeneity = .48).

Using the combined cohort and case-control data, when we examined associations within subgroups defined by factors that increase ovarian cancer risk, we observed possible effect modification by history of endometriosis (Fig 1). Among women without endometriosis, frequent aspirin use was associated with reduced ovarian cancer risk (RR, 0.82; 95% CI, 0.73 to 0.92), whereas no risk reduction was observed among women with endometriosis (RR, 1.15; 95% CI, 0.80 to 1.65; *P*-heterogeneity = .08). However, the CI for the latter effect estimate was large because of the small number of women with endometriosis (prevalence range, 1%-9% in the cohort studies, 3%-11% among OCAC controls). Frequent aspirin use was associated with lower ovarian cancer risk regardless of obesity, although the association was slightly stronger among obese women (RR, 0.79; 95% CI, 0.67 to 0.93) than among nonobese women

TABLE 1. Summary Relative Risks for the Association Between Frequent Aspirin Use and Ovarian Cancer Risk in OC3 and OCAC, by Subgroups Defined by Age at Study Enrollment

Age, years	No. of Studies Included	I^2 %	RR	95% CI	*P* (heterogeneity by study design)	*P* (heterogeneity by subgroup)
Cohort studies						
< 50	2	0.0	0.99	0.49 to 2.00	—	.78
50-59	9	0.0	0.87	0.69 to 1.09	—	
60-69	8	0.0	0.88	0.76 to 1.02	—	
≥ 70	6	26.7	1.05	0.76 to 1.46	—	
Case-control studies						
< 50	7	9.7	1.11	0.76 to 1.63	—	.26
50-59	8	46.5	0.91	0.66 to 1.24	—	
60-69	8	0.0	0.81	0.67 to 0.97	—	
≥ 70	8	0.0	0.72	0.57 to 0.91	—	
All studies						
< 50	9	0.0	1.09	0.79 to 1.49	.77	.56
50-59	17	0.0	0.87	0.75 to 1.02	.82	
60-69	16	0.0	0.85	0.76 to 0.95	.46	
≥ 70	14	23.8	0.86	0.69 to 1.06	.07	

NOTE. Models were adjusted for age, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use, and BMI.
Abbreviations: BMI, body mass index; OC3, Ovarian Cancer Cohort Consortium; OCAC, Ovarian Cancer Association Consortium; RR, relative risk.

(RR, 0.91; 95% CI, 0.80 to 1.03; *P*-heterogeneity = .18). Associations were similar across strata of family history of breast or ovarian cancer (RR, 0.86; 95% CI, 0.79 to 0.94 for women without a family history, RR, 0.88; 95% CI, 0.72 to 1.06 for women with a family history, *P*-heterogeneity = .88).

Consistent risk reductions were observed within subgroups defined by protective factors for ovarian cancer, including parity (*P*-heterogeneity = .71), duration of oral contraceptive use (*P*-heterogeneity = .79), and tubal ligation (*P*-heterogeneity = .27, Fig 1). There was also no effect modification by nonaspirin NSAID use (RR, 0.86; 95% CI, 0.77 to 0.95 for no NSAID use, RR, 0.86; 95% CI, 0.75 to 0.98 for NSAID use, *P*-heterogeneity = .95).

We did not observe effect modification by age at enrollment in the cohort (*P*-heterogeneity = .78) or case-control (*P*-heterogeneity = .26) studies (Table 1). However, in the case-control studies, there was possible strengthening of the association with age, with the strongest inverse association observed among women age 70 years or older at diagnosis/enrollment (OR, 0.72; 95% CI, 0.57 to 0.91).

In general, associations with frequent aspirin use were similar for all ovarian cancer histotypes (Fig 2, Appendix Table A3, online only). Risk reductions were particularly robust for high-grade serous ovarian cancer, both overall (RR, 0.86; 95% CI, 0.78 to 0.94) and across subgroups defined by ovarian cancer risk factors. For women with endometriosis, although there was no association between frequent aspirin use and ovarian cancer overall, there was suggestion of an inverse association with endometrioid ovarian cancer, the histotype most strongly associated with endometriosis.

In the cohort studies, 21% of women had none of the six ovarian cancer risk factors, 42% had one risk factor, and 37% had ≥ two. In the case-control studies, the corresponding percentages of women with zero, one, and ≥ two risk factors were 8%, 28%, and 64% for cases, and 12%, 37%, and 51% for controls. In analyses stratified by the number of risk factors (ie, the ovarian cancer risk score), frequent aspirin use was inversely associated with ovarian cancer risk among women at higher risk of ovarian cancer because of the presence of ≥ two risk factors (RR, 0.81; 95% CI, 0.73 to 0.90, Fig 1). The protective association for these higher-risk women was consistent across histotypes (Fig 2, Appendix Table A3, *P*-heterogeneity = .42). Among the higher-risk women, the NNT to prevent one ovarian cancer within 10 years was 970 (95% CI, 683 to 1,843, Appendix Table A4, online only). By contrast, the NNT for all women in the study population regardless of risk score was 1784 (95% CI, 1,160 to 3,866).

DISCUSSION

In this analysis of data from two ovarian cancer consortia, frequent aspirin use was associated with a 13% reduction in ovarian cancer risk overall. A similar risk reduction was observed for high-grade serous ovarian cancer, the most common and one of the most fatal histotypes, which is important because most established risk factors are more weakly associated with high-grade serous ovarian cancers.[6] The consistency of the frequent aspirin use-ovarian cancer association across the individual case-control and cohort study populations was notable and provides strong

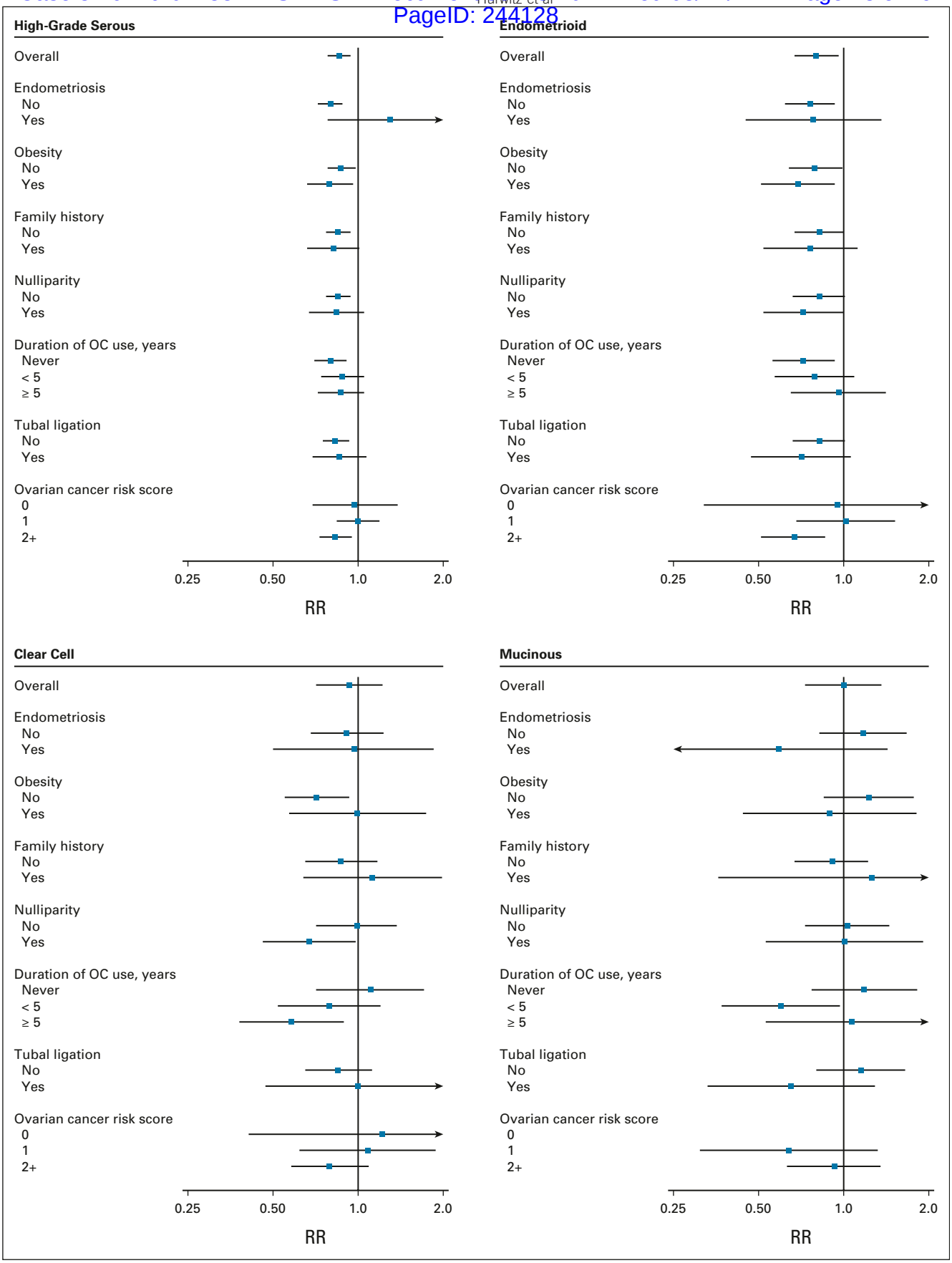

FIG 2. (Continued).

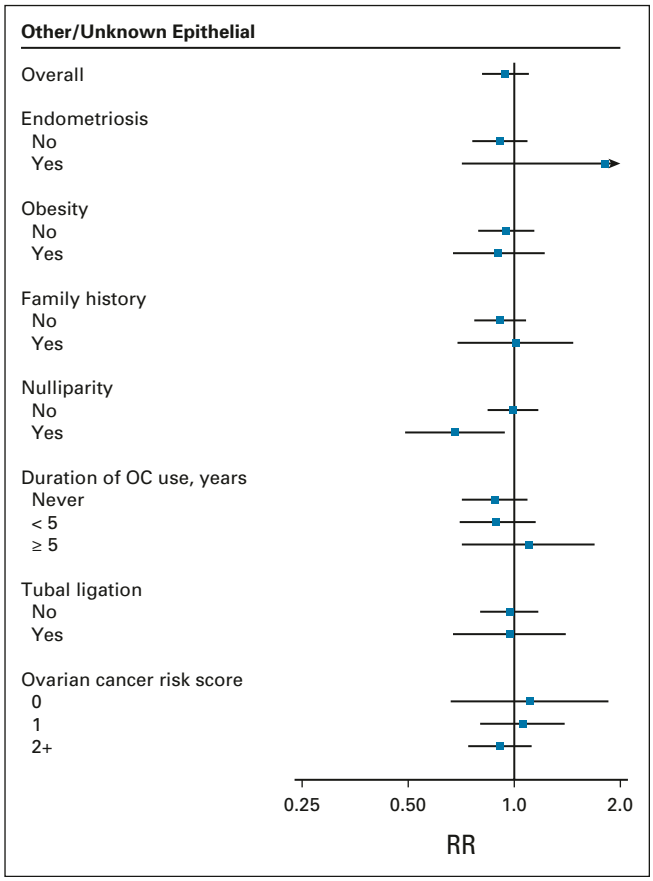

FIG 2. (Continued). Summary RRs for the associations between frequent aspirin use and each ovarian cancer histotype in OC3 and OCAC, overall and by key subgroups of interest. Tests for heterogeneity in the association across ovarian cancer histotypes: overall (P = .60), no endometriosis (P = .17), endometriosis (P = .31), no obesity (P = .13), obesity (P = .69), no family history of breast/ovarian cancer (P = .93), family history of breast/ovarian cancer (P = .64), parous (P = .39), nulliparous (P = .64), no OC use (P = .19), < 5 years of OC use (P = .62), 5+ years of OC use (P = .27), no tubal ligation (P = .35), tubal ligation (P = .74), ovarian cancer risk score = 0 (P = .96), ovarian cancer risk score = 1 (P = .79), and ovarian cancer risk score ≥ 2 (P = .42). Models were adjusted for age, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use, BMI, and study. Models stratified by risk score were adjusted for age, duration of menopausal hormone therapy use, and study. For mucinous ovarian cancers, the RR for women with ovarian cancer risk score = 0 was unable to be estimated. BMI, body mass index; OC, oral contraceptive; OC3, Ovarian Cancer Cohort Consortium; OCAC, Ovarian Cancer Association Consortium; RR, relative risk.

support for a beneficial effect of frequent aspirin use on ovarian cancer risk.

Importantly, our study also found that established ovarian cancer risk factors do not modify the association between frequent aspirin use and ovarian cancer risk. There was a suggestion of effect modification by endometriosis, with an inverse association observed for women without but not with self-reported endometriosis, but this was likely driven by the small number of women with endometriosis and the limited power to detect associations within this subgroup. Additionally, there was no effect modification by endometriosis for endometrioid or clear cell tumors, the two specific histotypes for which endometriosis is a risk factor.[6]

Risk reductions associated with frequent aspirin use were otherwise consistent across subgroups defined by factors that increase (obesity and family history of breast/ovarian cancer) and decrease (parity, oral contraceptive use, and tubal ligation) ovarian cancer risk. The lack of effect modification by adiposity is particularly notable, given that other studies have reported aspirin to be more weakly associated

with reduced cardiovascular disease and colorectal cancer risk[55,56] and more strongly associated with reduced endometrial cancer risk[57] among obese individuals; this could suggest that aspirin's mechanism of action for preventing cardiovascular disease and these other cancers may differ from that preventing ovarian cancer.

There was possible effect modification by the ovarian cancer risk score, with a null association observed among women with zero ovarian cancer risk factors. However, the results for women with zero risk factors were inconclusive, given the small number of cases and heterogeneity in the study-specific results for this subgroup. More critically, we observed a clear inverse association between frequent aspirin use and ovarian cancer among women with multiple ovarian cancer risk factors. These results are important, given that any implementation of aspirin use for ovarian cancer chemoprevention will likely need to focus on specific high-risk subgroups.[28] Our study suggests that frequent aspirin use is protective among women at increased risk of ovarian cancer because of the presence of established epidemiologic risk factors, with a lower NNT among women with ≥ 2 risk factors, and that targeting chemoprevention programs to women with epidemiologic risk factors may thus be a viable strategy.

To our knowledge, this study is the largest to date on aspirin and ovarian cancer risk and the first to examine effect modification by a comprehensive set of ovarian cancer risk factors. Previous studies of aspirin, examined alone or combined with other NSAIDs, have also reported no effect modification by BMI,[17,18] parity,[12,16,18,46] or oral contraceptive use,[16,18,46] but these individual studies were only powered to detect very strong differences. One study observed a possible stronger association between daily aspirin use and ovarian cancer risk with increasing BMI,[58] a trend that was mirrored in our study, although our study suggests that frequent aspirin may still be modestly protective among nonobese women. Our study confirms and expands upon these prior studies by combining the existing observational data, which facilitated a well-powered analysis, even among subgroups. Access to the individual-level data from each study allowed us to apply a standardized analytic approach, assess associations by histotype, and focus specifically on frequent aspirin use, the pattern of use that appears most protective against ovarian cancer.[25,26]

Although we combined results across study design, such pooling was necessary to obtain sufficient power to test for effect modification. Moreover, formal comparison of the cohort and case-control results revealed no meaningful or statistically significant differences. There may have been some bias because of the use of observational data, but research has found that observational studies of aspirin and cancer can recapitulate randomized controlled trial findings when there is detailed recording of aspirin use and careful adjustment for confounders.[59] We were unable to examine associations specifically for low-dose aspirin, which has been more strongly associated with reduced ovarian cancer risk in prior studies,[13,26] but frequent aspirin use was highly correlated with low-dose use in the studies with dosage information available ($\rho = 0.97$ in OC3, $\rho = 0.82$ in OCAC controls). We did not have data on indication for aspirin use or age at initiation of use, both of which require further study. We did not look at associations among women at increased risk of ovarian cancer because of common or rare genetic variants as genetic data were not available for all included studies; whether aspirin reduces risk among women with highly penetrant mutations (ie, BRCA1/BRCA2 or Lynch syndrome) will require examination in specialized study populations. Finally, when calculating the NNT, we were unable to incorporate associations for precise durations of aspirin use because of the use of observational data. The NNT also does not account for the known risks associated with frequent aspirin use, and further research on the net benefits and harms is needed.

In conclusion, this study, the largest to date on frequent aspirin use and ovarian cancer, supports a 13% reduction in ovarian cancer risk with frequent aspirin use, with a 14% reduction for high-grade serous carcinoma, the most common and one of the more lethal histotypes. Similar risk reductions were observed across subgroups defined by established epidemiologic risk factors, and no subgroup experienced a significant increased risk with aspirin use. These results suggest that primary prevention of ovarian cancer is an added benefit of frequent aspirin use that could be incorporated into composite risk-benefit calculations. Given that women with elevated ovarian cancer risk because of epidemiologic risk factors also benefit and that the NNT to prevent one ovarian cancer is lower for higher-risk women, future work should explore how chemoprevention programs with aspirin could complement existing preventive strategies, which are currently limited to women with the highest risk (ie, prophylactic salpingo-oophorectomy for BRCA1/2 carriers) and target additional high-risk subgroups to maximize population-level impact and minimize risks.

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DISCLAIMER

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SUPPORT

This study was funded by US Department of Defense Ovarian Cancer Research Program (W81XWH-19-1-0346). The OC3 received support from the US Department of Defense Ovarian Cancer Research Program (W81XWH-12-1-0561, W81XWH-19-1-0346) and the Intramural Research Program of the National Cancer Institute at the National Institutes of Health. The Ovarian Cancer Association Consortium was supported by a grant from the Ovarian Cancer Research Fund thanks to donations by the family and friends of Kathryn Sladek Smith. The Australian Ovarian Cancer Study and Australian Cancer Study were funded by the US Army Medical Research and Material Command (DAMD17-01-1-0729), National Health and Medical Research Council of Australia (199600, 400413), Cancer Councils of New South Wales, Victoria, Queensland, South Australia, and Tasmania, Cancer Foundation of Western Australia. All aspects of the Cancer Prevention Study II were funded by the Intramural Research Program of the American Cancer Society and by the National Cancer Institute at the National Institutes of Health Intramural Research Program. The California Teachers Study and the research reported in this publication were supported by National Cancer Institute at the National Institutes of Health (grant numbers U01 CA199277, P30 CA033572, P30 CA023100, UM1 CA164917, and R01 CA077398). The Diseases of the Ovary and their Evaluation Study was funded by R01 CA112523 and R01 CA87538. The Hawaii Ovarian Cancer Case–Control Study was funded by R01 CA58598, N01 CN55424, and N01 PC67001. The Hormones and Ovarian Cancer Prediction Study was funded by R01 CA95023 and Department of Defense (DOD) grant DAMD17-02-1-0669. The Iowa Women's Health Study was funded by the National Cancer Institute at the National Institutes of Health (R01 CA39742). The North Carolina Ovarian Cancer Study was funded by R01 CA76016 and the DOD grant DAMD17-02-1-0666. The Nurses' Health Study and Nurses' Health Study II were funded by the National Cancer Institute at the National Institutes of Health (UM1 CA186107, P01 CA87969, R01 CA49449, U01 CA176726, and R01 CA67262). The NIH-AARP Diet and Health Study was funded by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health. PLCO was funded through contracts administered by the Division of Cancer Prevention at the National Cancer Institute, National Institutes of Health, with support from the National Cancer Institute Intramural Research Program. The Sister Study was funded by Intramural Research Program of the National Institute of Environmental Health Sciences at the National Institutes of Health (Z01ES044005). The University of California, Irvine Ovarian Cancer Study was funded by R01 CA58860, R01 CA92044, PSA 042205, and the Lon V Smith Foundation grant LVS-39420. The United Kingdom Ovarian Cancer Population Study was funded by Cancer Research UK, the Eve Appeal, and the OAK Foundation. The University of Southern California, Study of Lifestyle and Women's Health was funded by R01 CA17054, R01 CA14089, R01 CA61132, N01-PC-67010, P01 CA17054, California Cancer Research Program (00-01389V-20170, R03 CA113148, R03 CA115195, N01 CN25403), and California Cancer Research Program (2II0200; USC). The Vitamins and Lifestyle Study was funded by the National Cancer Institute and Office of Dietary Supplements at the National Institutes of Health (K05 CA154337).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.21.01900.

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Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

ACKNOWLEDGMENT

The authors would like to thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, and WY. The authors assume full responsibility for analyses and interpretation of these data. The authors would like to acknowledge the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, as the home of the Nurses' Health Study. The collection of cancer incidence data used in the California Teachers Study was supported by the California Department of Public Health pursuant to California Health and Safety Code Section 103885; Centers for Disease Control and Prevention's National Program of Cancer Registries, under cooperative agreement 5NU58DP006344; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201800032I awarded to the University of California, San Francisco, contract HHSN261201800015I awarded to the University of Southern California, and contract HHSN261201800009I awarded to the Public Health Institute. The opinions, findings, and conclusions expressed herein are those of the author(s) and do not necessarily reflect the official views of the State of California, Department of Public Health, the National Cancer Institute, the National Institutes of Health, the Centers for Disease Control and Prevention or their contractors and subcontractors, or the regents of the University of California, or any of its programs.

The Australian Ovarian Cancer Study Management Group (D. Bowtell, G. Chenevix-Trench, A. deFazio, D. Gertig, A. Green, and P. Webb) and Australian Cancer Study investigators (A. Green, P. Parsons, N. Hayward, P. Webb, and D. Whiteman) thank all the clinical and scientific collaborators (see http://www.aocstudy.org/) and the women for their contribution. Some of this work was undertaken at University College London Hospital/University College London, which received a proportion of funding from the Department of Health's National Institutes for Health Research Biomedical Research Centre funding scheme. The authors particularly thank I. Jacobs, M. Widschwendter, E. Wozniak, A. Ryan, J. Ford, and N. Balogun for their contribution to the study. Support for title page creation and format was provided by AuthorArranger, a tool developed at the National Cancer Institute. AOCS Study Group members are listed at Appendix 1 (online only).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Modification of the Association Between Frequent Aspirin Use and Ovarian Cancer Risk: A Meta-Analysis Using Individual-Level Data From Two Ovarian Cancer Consortia

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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Research Funding: GRAIL (Inst)

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Patents, Royalties, Other Intellectual Property: Patent related treatment of PAD (peripheral arterial disease)(I)

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No other potential conflicts of interest were reported.

Published by American Society of Clinical Oncology

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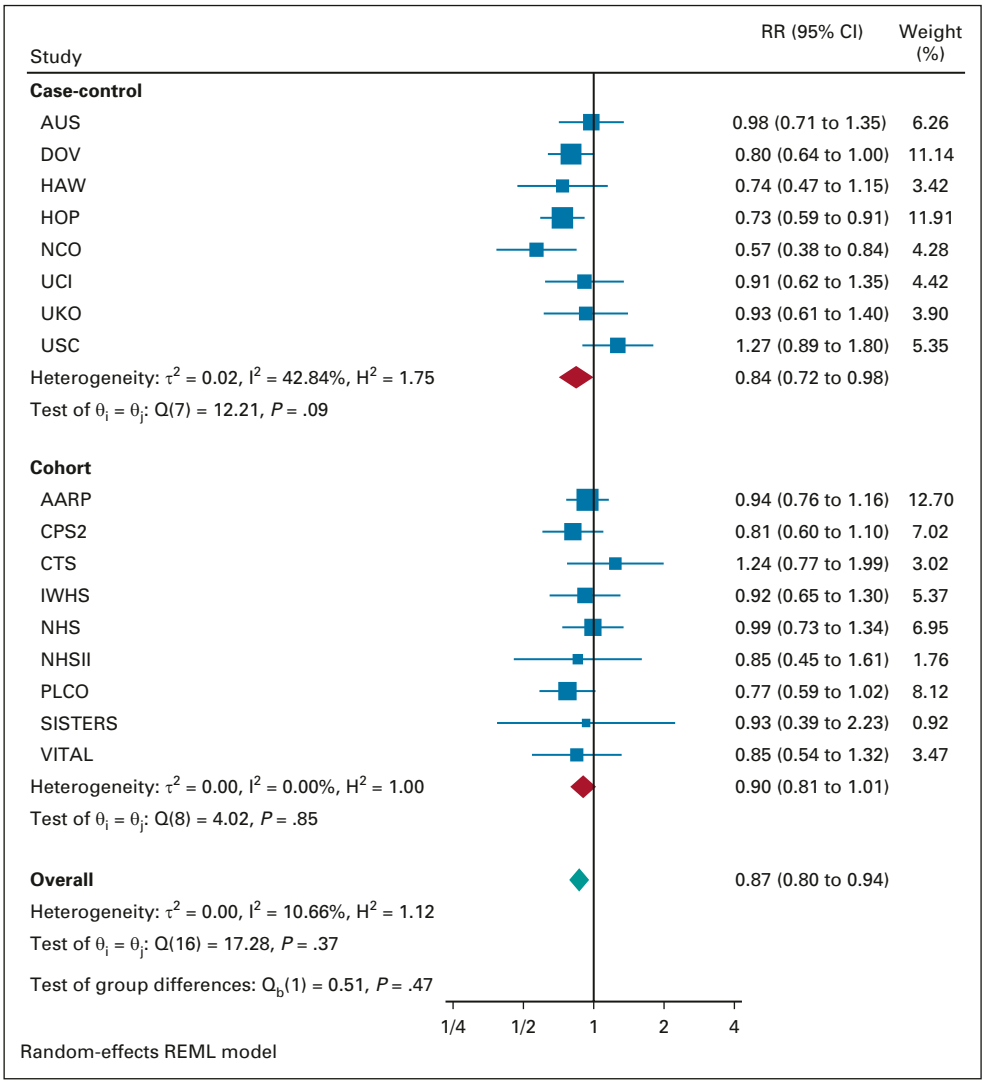

FIG A1. Meta-analysis of the overall association[a] between frequent aspirin use and ovarian cancer risk in OC3 and OCAC. [a]Adjusted for age, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use, and BMI. AARP, NIH-AARP Diet and Health Study; AUS, Australian Ovarian Cancer Study & Australian Cancer Study; BMI, body mass index; CPS2, Cancer Prevention Study II Nutrition Cohort; CTS, California Teachers Study, DOV, Diseases of the Ovary and their Evaluation Study; HAW, Hawaii Ovarian Cancer Study; HOP, Hormones and Ovarian Cancer Prediction Study; IWHS, Iowa Women's Health Study; NCO, North Carolina Ovarian Cancer Study; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; OC3, Ovarian Cancer Cohort Consortium; OCAC, Ovarian Cancer Association Consortium; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; RR, relative risk; SISTERS, Sister Study; UCI, University of California, Irvine Ovarian Cancer Study; UKO, United Kingdom Ovarian Cancer Population Study; USC, University of Southern California, Study of Lifestyle and Women's Health; VITAL, Vitamins and Lifestyle Cohort.

TABLE A1. Characteristics of the Included Cohort Studies From Ovarian Cancer Cohort Consortium

Study	Acronym	Location	Baseline Enrollment Period[a]	Questionnaire Item for Aspirin Use	Categories for Frequency of Use	No. at Risk	No. of Events	Average Follow-Up,[a] years (max)	Average Age at Entry,[b] years	Prevalence of Frequent Aspirin Use
NIH-AARP Diet and Health Study	AARP	United States	1995-1996	During the past 12 months, did you take any of the following aspirin products?	< 2/month, 2-3/month, 1-2/week, 3-4/week, 5-6/week, 1/day, ≥ 2/day	98,367	649	9.8 (11.2)	61.9	19.9%
Cancer Prevention Study II Nutrition Cohort	CPS2	United States	1992-1993	During the past year, did you take any of the following medications regularly?	Fill in times per month, pills per day	63,380	538	13.8 (16.7)	62.0	14.3%
California Teachers Study	CTS	United States	1995	Have you taken any of the following medications regularly (at least once a week)? If so, indicate how many total years you took it and how often you took it	1-3, 4-6, every day	43,782	185	14.3 (15.2)	51.8	9.8%
Iowa Women's Health Study	IWHS	United States	1986	On average, how often do you take aspirin?	Never, < 1/week, 1/week, 2-5/week, 6-7/week, 8-14/week, 15+/week	23,269	222	14.0 (16.2)	68.2	38.0%
Nurses' Health Study	NHS	United States	1976	Mark if used regularly in the past 2 years	Days/week: 1, 2-3, 4-5, 6+; Tablets/week: 1-2, 3-5, 6-14, 15+ tablets	58,357	339	9.2 (10.0)	65.8	35.8%
Nurses' Health Study II	NHSII	United States	1989	Mark if used regularly in the past 2 years	Days/week: 1, 2-3, 4-5, 6+; Tablets/week: 1-2, 3-5, 6-14, 15+ tablets	77,235	137	9.7 (10.0)	46.0	10.9%
Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	PLCO	United States	1993-2002	During the past 12 months, have you regularly used aspirin or aspirin-containing products?	1/day, 2+/day, 1/week, 2/week, 3-4/week, < 2/month, 2-3/month	60,144	363	11.9 (17.0)	62.5	29.3%
Sister Study	SISTERS	United States	2003-2009	Do you currently take any prescription or nonprescription medications at least once a week? Also captured information in a grid-format to ascertain lifetime medication usage	Fill in days per week, times per day	39,195	39	4.6 (8.1)	54.7	20.6%

(continued on following page)

TABLE A1. Characteristics of the Included Cohort Studies From Ovarian Cancer Cohort Consortium (continued)

Study	Acronym	Location	Baseline Enrollment Period[a]	Questionnaire Item for Aspirin Use	Categories for Frequency of Use	No. at Risk	No. of Events	Average Follow-Up,[a] years (max)	Average Age at Entry,[b] years	Prevalence of Frequent Aspirin Use
Vitamins and Lifestyle Cohort	VITAL	United States	2000-2002	In the past 10 years, did you take any of the following medications at least one per week for a year?	1-3, 4-6, 7 days/week	27,922	128	9.4 (11.2)	61.3	25.5%

[a]Follow-up time for this analysis began accruing at the time of the questionnaire collecting information on frequency of aspirin use (AARP: 1996-1997; IWHS: 1992; NHS: 2000-2001; NHSII: 2001-2002).

[b]Age at the time of the questionnaire collecting information on frequency of aspirin use.

TABLE A2. Characteristics of the Included Case-Control Studies From Ovarian Cancer Association Consortium

Study	Acronym	Location	Ascertainment Period	Cases	Controls	Questionnaire Item for Aspirin Use	Categories for Frequency of Use	Average Age at Entry for Cases, years	Prevalence of Frequent Aspirin Use Among Controls (%)
Australian Ovarian Cancer Study & Australian Cancer Study	AUS	Australia	2002-2006	1,311	1,505	How often have you taken the following over-the-counter (aspirin, paracetamol, anti-inflammatory drugs) medications during the PAST 5 years?	Never, occasionally, < 1/month, 1/week, 2-3/week, 4-7/week, 2+/day	59.3	6.2
Diseases of the Ovary and their Evaluation Study	DOV	United States	2002-2009	1,159	1,849	Before the reference date, have you taken any of these medications (show card) 5 or more days per month for at least 6 months?	Days per month: 5-7, 8-14, > 14 days but less than daily, daily, or almost daily	56.2	14.9
Hawaii Ovarian Cancer Study	HAW	United States	2001-2008	256	485	Did you ever take an aspirin product (show card) at least 12 times a year?	No. of pills taken per day, week, or month	56.9	19.2
Hormones and Ovarian Cancer Prediction Study	HOP	United States	2003-2008	683	1,513	Before reference date have you ever used aspirin (show card) for at least two tablets per week continuously for a period of 6 months or longer?	No. of pills taken per day, week, or month	60.2	29.8
North Carolina Ovarian Cancer Study	NCO	United States	1999-2008	939	1,085	For the 5 years before diagnosis, did you take any of these over-the-counter medications (show card) on a regular basis for at least 3 months?	Days per month: ≤ 1, 2-7, 8-14, > 14, daily or almost daily	57.2	9.0

(continued on following page)

TABLE A2. Characteristics of the Included Case-Control Studies From Ovarian Cancer Association Consortium (continued)

Study	Acronym	Location	Ascertainment Period	Cases	Controls	Questionnaire Item for Aspirin Use	Categories for Frequency of Use	Average Age at Entry for Cases, years	Prevalence of Frequent Aspirin Use Among Controls (%)
University of California, Irvine Ovarian Cancer Study	UCI	United States	1995-2005	393	313	Have you taken medication listed (aspirin, ibuprofen, acetaminophen, and naproxen) regularly? By regular, we are referring to use of the drug or medication at least once a week for a year, or more than 50 pills during a one year-period	No. of pills/week	58.0	5.6
United Kingdom Ovarian Cancer Population Study	UKO	United Kingdom	2006-2007	516	598	Have you ever used any medication containing the drugs (aspirin or ibuprofen) on a regular basis (by regular, we mean every day or almost every day for 6 months or longer)?	Every day or almost every day	60.7	15.2
University of Southern California, Study of Lifestyle and Women's Health	USC	United States	2000-2005	469	679	Before reference date, as an adult, did you ever take any prescription or nonprescription medicine at least 2 or more times per week for one month or longer?	No. of days/month	57.0	12.7

TABLE A3. Summary RRs and 95% CIs for the Associations Between Frequent Aspirin Use and Each Ovarian Cancer Histotype in OC3 and OCAC, Overall and by Key Subgroups of Interest

Subgroup	High-Grade Serous, RR (95% CI)	Endometrioid, RR (95% CI)	Clear Cell, RR (95% CI)	Mucinous, RR (95% CI)	Other/Unknown Epithelial, RR (95% CI)	*P*-Heterogeneity
Overall	0.86 (0.78 to 0.94)	0.80 (0.67 to 0.96)	0.93 (0.71 to 1.22)	1.00 (0.73 to 1.36)	0.94 (0.81 to 1.10)	.60
Endometriosis						
No	0.80 (0.72 to 0.88)	0.76 (0.62 to 0.93)	0.91 (0.68 to 1.23)	1.17 (0.82 to 1.67)	0.91 (0.76 to 1.09)	.17
Yes	1.30 (0.78 to 2.16)	0.78 (0.45 to 1.36)	0.97 (0.50 to 1.85)	0.59 (0.24 to 1.43)	1.84 (0.71 to 4.78)	.31
Obesity						
No	0.87 (0.78 to 0.98)	0.79 (0.64 to 0.99)	0.71 (0.55 to 0.93)	1.23 (0.85 to 1.77)	0.95 (0.79 to 1.14)	.13
Yes	0.79 (0.66 to 0.96)	0.69 (0.51 to 0.93)	0.99 (0.57 to 1.74)	0.89 (0.44 to 1.81)	0.90 (0.67 to 1.22)	.69
Family history of breast/ ovarian cancer						
No	0.85 (0.77 to 0.94)	0.82 (0.67 to 1.00)	0.87 (0.65 to 1.17)	0.91 (0.67 to 1.22)	0.91 (0.77 to 1.08)	.93
Yes	0.82 (0.66 to 1.01)	0.76 (0.52 to 1.12)	1.12 (0.64 to 1.98)	1.26 (0.36 to 4.41)	1.01 (0.69 to 1.47)	.64
Nulliparity						
No	0.85 (0.77 to 0.94)	0.82 (0.66 to 1.01)	0.99 (0.71 to 1.37)	1.03 (0.73 to 1.45)	0.99 (0.84 to 1.17)	.39
Yes	0.84 (0.67 to 1.05)	0.72 (0.52 to 1.00)	0.67 (0.46 to 0.98)	1.01 (0.53 to 1.91)	0.68 (0.49 to 0.94)	.64
Duration of OC use, years						
Never	0.80 (0.70 to 0.91)	0.72 (0.56 to 0.93)	1.11 (0.71 to 1.71)	1.18 (0.77 to 1.82)	0.88 (0.71 to 1.09)	.19
< 5	0.88 (0.74 to 1.05)	0.79 (0.57 to 1.09)	0.79 (0.52 to 1.20)	0.60 (0.37 to 0.97)	0.89 (0.70 to 1.15)	.62
≥ 5	0.87 (0.72 to 1.05)	0.96 (0.65 to 1.41)	0.58 (0.38 to 0.89)	1.07 (0.53 to 2.19)	1.10 (0.71 to 1.69)	.27
Tubal ligation						
No	0.83 (0.75 to 0.93)	0.82 (0.66 to 1.01)	0.85 (0.65 to 1.12)	1.15 (0.80 to 1.65)	0.97 (0.80 to 1.17)	.35
Yes	0.86 (0.69 to 1.07)	0.71 (0.47 to 1.06)	1.00 (0.47 to 2.15)	0.65 (0.33 to 1.29)	0.97 (0.67 to 1.40)	.74
Ovarian cancer risk score						
0	0.97 (0.69 to 1.38)	0.95 (0.32 to 2.84)	1.22 (0.41 to 3.65)		1.11 (0.66 to 1.85)	.96
1	1.00 (0.84 to 1.19)	1.02 (0.68 to 1.52)	1.08 (0.62 to 1.88)	0.64 (0.31 to 1.32)	1.06 (0.80 to 1.39)	.79
2+	0.83 (0.73 to 0.95)	0.67 (0.51 to 0.86)	0.79 (0.58 to 1.09)	0.93 (0.63 to 1.35)	0.91 (0.74 to 1.12)	.42

NOTE. Models were adjusted for age, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use, BMI, and study. Models stratified by risk score were adjusted for age, duration of menopausal hormone therapy use, and study. For mucinous ovarian cancers, the relative risk for women with ovarian cancer risk score = 0 was unable to be estimated.

Abbreviations: BMI, body mass index; OC, oral contraceptive; OC3, Ovarian Cancer Cohort Consortium; OCAC, Ovarian Cancer Association Consortium; RR, relative risk.

TABLE A4. NNT With Frequent Aspirin Use to Prevent One Incident Ovarian Cancer Within 10 Years, Overall and by the Ovarian Cancer Risk Score

Subgroup	No. of Cases From Cohort Studies	No. of Cases From Case-Control Studies	10-Year Cumulative Incidence of Ovarian Cancer in Nonaspirin Users[a]	RR (95% CI)[b]	NNT (95% CI)[c]
Overall	2,600	5,726	0.00432	0.87 (0.80 to 0.94)	1,784 (1,160 to 3,866)
Ovarian cancer risk score					
0	447	438	0.00343	0.97 (0.79 to 1.19)	9,735 (1,391 to ∞)[d]
1	943	1,377	0.00481	0.93 (0.82 to 1.06)	2,977 (1,158 to ∞)[d]
2+	1,151	3,104	0.00544	0.81 (0.73 to 0.90)	970 (683 to 1,843)

Abbreviations: NNH, number needed to harm; NNT, number needed to treat; RR, relative risk.

[a]Calculated using the pooled cohort study data.

[b]Combined cohort and case-control summary RRs for the association between frequent aspirin use and ovarian cancer risk.

[c]$NNT = 1/(S(t)^{RR} - S(t))$, where S(t) = 1- to 10-year cumulative incidence of ovarian cancer in nonaspirin users.[54]

[d]Given that the 95% CI for the RR overlaps 1, we cannot preclude the possibility that frequent aspirin use is associated with harm (ie, the full 95% CI extends to include the possibility of a positive NNH).[60]

Exhibit 116

Effects of risk factors for ovarian cancer in women with and without endometriosis

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Received January 11, 2022; revised July 16, 2022; accepted July 19, 2022.

M.T.P. has nothing to disclose. A.M. reports consulting fees paid to their institution from AstraZeneca and Pfizer as well as reports stock and stock options ownership of Clovis Oncology. B.T. has nothing to disclose. P.M.W. reports grant funding to their institution from AstraZeneca, US Army Medical Research and Materiel Command, National Health and Medical Research Council of Australia, Cancer Councils of New South Wales, Victoria, Queensland, South Australia, and Tasmania and Cancer Foundation of Western Australia as well as receipt of a speaker's fee from AstraZeneca. S.J.J. has nothing to disclose. K.L.T. has nothing to disclose. D.W.C. reports payment for expert testimony from Ferraro Law Firm and Ashcraft and Gerel Law Firm and grant funding to their institution from the National Institutes of Health. L.J.T. reports grant funding to their institution from the National Cancer Institute. H.A.R. has nothing to disclose. J.A.D. has nothing to disclose. H.R.H. has nothing to disclose. M.T.G. has nothing to disclose. F.M. reports receipt of grants to their institution from the National Cancer Institute, the Department of Defense, and the Deans Faculty Advancement Fund and National Science Foundation. K.B.M. has nothing to disclose. A.J. has nothing to disclose. S.K.K. reports grant funding to their institution from Mermaid Project (Mermaid 3). H.A. has nothing to disclose. A.Z. has nothing to disclose. A.B. has nothing to disclose. L.K. has nothing to disclose. A.H.W. has nothing to disclose. M.C.P reports grant funding to their institution from the National Cancer Institute and the Department of Defense. C.L.P. reports grant funding to their institution from the National Institutes of Health and the Department of Defense; receipt of reimbursements for travel expenses from the Canadian Conference on Ovarian Cancer Research; declining an honorarium for participation on an advisory board for the Ovarian Cancer Research Alliance; and no payment received for a leadership role in the Ovarian Cancer Association Consortium. A.W.L. reports grant funding to their institution from the Rivkin Center for Ovarian Cancer.

M.T.P. and A.M. should be considered similar in author order.

The Ovarian Cancer Association Consortium (OCAC) was funded by the generous contributions of its research investigators. It was also supported in part by the National Cancer Institute in Bethesda, US GAME-ON Post-GWAS Initiative (U19-CA148112). This study made use of data generated by the Wellcome Trust Case Control consortium that was supported by the Wellcome Trust in London, UK (award 076113). The AUS study was supported by the Army Medical Research and Materiel Command Department of Defense in Fort Detrick, US (DAMD17-01-1-0729), National Health & Medical Research Council in Canberra, Australia (199600, 400413, 400281), Cancer Councils of New South Wales, Victoria, Queensland, South Australia, and Tasmania and Cancer Foundation of Western Australia (Multi-State Applications 191, 21, 182). The AUS study was also supported by Ovarian Cancer Australia in Melbourne, Australia and the Peter MacCallum Foundation in Melbourne, Australia. P.M.W. was supported by an Investigator Grant from the National Health & Medical Research Council in Canberra, Australia (APP1173346). The CON study was supported by the National Institutes of Health in Bethesda, US (R01-CA063678, R01-CA074850, R01-CA080742). The DOV study was supported by the National Institutes of Health in Bethesda, US (R01-CA112523, R01-CA87538). The HAW study was supported by the National Institutes of Health in Bethesda, US (R01-CA58598, N01-CN55424, N01-PC67001). The HOP study was supported by the Army Medical Research and Materiel Command Department of Defense in Fort Detrick, US (DAMD17-02-1-0669), the National Cancer Institute in Bethesda, US (K07-CA080668, R01-CA95023), and the National Institutes of Health/National Center for Research Resources/General Clinical Research Center in Bethesda, US (M01-RR000056). The MAL study was supported by the National Cancer Institute at the National Institutes for Health in Bethesda, US (R01-CA61107), the Danish Cancer Society in Copenhagen, Denmark (94 222 52), and the Mermaid I and III projects in Copenhagen, Denmark. The NEC study was supported by the National Institutes of Health in Bethesda, US (R01-CA54419, P50-CA105009), and the Army Medical Research and Materiel Command Department of Defense in Fort Detrick, US (W81XWH-10-1-02802). The UCI study was supported by the National Institutes of Health in Bethesda, US (R01-CA058860) and the Lon V Smith Foundation in Beverly Hills, US (LVS-39420). The USC study was supported by the National Institutes of Health in Bethesda, US (P01-CA17054, P30-CA14089, R01-CA61132, N01-PC67010, R03-CA113148, R03-CA115195, N01-CN025403) and the California Cancer Research Program in the US (00-01389V-20170, 2II0200). M.C.P. was partially supported by the National Institutes of Health/National Cancer Institute support grant to Memorial Sloan Kettering Cancer Center (PI: C.B. Thompson) (P30-CA008748). B.T. was partially supported in part by the National Institutes of Health/National Cancer Institute support grant to the Huntsman Cancer Institute (P30-CA040214).

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Fertility and Sterility® Vol. ■, No. ■, ■ 2022 0015-0282

https://doi.org/10.1016/j.fertnstert.2022.07.019

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Objective: To evaluate the associations between 10 well-established ovarian cancer risk factors and risk of ovarian cancer among women with vs. without endometriosis.
Design: Pooled analysis of 9 case-control studies in the Ovarian Cancer Association Consortium.
Setting: Population-based.
Patient(s): We included 8,500 women with ovarian cancer, 13,592 control women.
Intervention(s): Ten well-established ovarian cancer risk factors.
Main Outcome Measure(s): Risk of ovarian cancer for women with and without endometriosis.
Result(s): Most risk factor-ovarian cancer associations were similar when comparing women with and without endometriosis, and no interactions were statistically significant. However, body mass index (BMI) 25–<30 kg/m^2 was associated with increased ovarian cancer risk among women with endometriosis (odds ratio [OR] = 1.27, 95% confidence interval [CI] 1.00–1.60), but not associated with the risk among women without endometriosis (OR = 0.97; 95% CI, 0.91–1.05) when compared with BMI 18.5–<25 kg/m^2; an increased risk was observed for a BMI ≥30 kg/m^2, although there was little difference comparing women with endometriosis (OR = 1.21; 95% CI, 0.94–1.57) to women without (OR = 1.13; 95% CI, 1.04–1.22) (*P*-interaction = .51). Genital talcum powder use and long-term menopausal estrogen-only therapy use showed increased ovarian cancer risk, but risk appeared greater for those with endometriosis vs. those without (genital talcum powder: OR = 1.38; 95% CI, 1.04–1.84 vs. OR = 1.12; 95% CI, 1.01–1.25, respectively; ≥10 years of estrogen-only therapy: OR = 1.88; 95% CI, 1.09–3.24 vs. OR = 1.42; 95% CI, 1.14–1.76, respectively); neither of these interactions were statistically significant (*P*-interaction = .65 and *P*-interaction = .96, respectively).
Conclusion(s): The associations between ovarian cancer and most risk factors were similar among women with and without endometriosis. However, there was some suggestion of differences by endometriosis status for BMI, menopausal hormone therapy use, and genital talcum powder use, highlighting the complexity of ovarian cancer etiology. (Fertil Steril® 2022;■:■–■. ©2022 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, ovarian cancer, effect modification, risk factors, interactions

Endometriosis is a common gynecologic condition that involves the growth of endometrial glands and stroma outside the uterine cavity (1). Its association with the risk of ovarian cancer is well established; there is a 3-fold increased risk for the clear cell histotype and a 2-fold increased risk for the endometrioid as well as low-grade serous histotypes (1, 2). In general, endometriosis and ovarian cancer are thought to have a shared pathophysiology (3), and there is also some evidence of a genetic link between these conditions (4, 5).

It has been suggested that the effects of ovarian cancer risk factors may be different among women with vs. without endometriosis. An Australian record-linkage study by Dixon-Suen et al. (6) and a pooled analysis of 11 case-control studies by Khoja et al. (7) found hysterectomy to be associated with a significantly reduced risk of ovarian cancer among women who had endometriosis, but to have no association among women who did not have endometriosis. Effect differences by history of endometriosis for other ovarian cancer risk factors are possible and should be evaluated, although to our knowledge only 1 study has done this.

Modugno et al. (8) considered the effect modification by endometriosis status and found no statistically significant differences possibly because of a small sample size (177 ovarian cancer cases with endometriosis, 184 controls with endometriosis). Thus, we conducted a comprehensive study of endometriosis as an effect modifier of ovarian cancer risk factors using epidemiologic data from over 22,000 women in the Ovarian Cancer Association Consortium (OCAC), of whom more than 800 cases and 900 controls had endometriosis. Our analysis considers 10 well-established ovarian cancer risk factors, including body mass index (BMI), talcum powder (i.e., talc) use, family history of ovarian cancer, nonsteroidal anti-inflammatory drug (NSAID) use, breastfeeding, hormonal oral contraceptive use, parity, tubal ligation, menopausal hormone therapy (HT) use (estrogen-only therapy and estrogen-progestin therapy), and age at menarche. We hypothesized that the associations between these factors and ovarian cancer risk may be different among women with and without endometriosis.

MATERIALS AND METHODS

Study Population

Data from 9 population based case-control studies were included in this pooled analysis; 1 study was conducted in

Australia (9), 1 in Denmark (10), and the remaining in the United States (11–17). These studies are part of the OCAC, an international collaboration that collects and shares risk factor data for the purposes of increasing power for analyses of genetic and environmental exposures (http://ocac.ccge.medschl.cam.ac.uk/). Cases were women with pathologically confirmed high-grade serous, low-grade serous, mucinous, endometrioid, clear cell, and other invasive epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnoses (hereafter referred to as ovarian cancer). Controls were women who had at least 1 ovary but had not been diagnosed with ovarian cancer on or before their reference date (i.e., date of interview at time of study enrollment). Details of each included study are summarized in Table 1. Overall, the study enrollment of cases and controls spanned from 1992 to 2010.

Across the 9 studies, 8,500 ovarian cancer cases and 13,592 control women self-reported whether they had a history of endometriosis and were thus included in the analysis (Table 1). Our study did have some overlap with the report by Modugno et al. (8) for participants ascertained from 1993 to 1999 for 2 OCAC studies: the Hawaii Ovarian Cancer Study (HAW) (approximately 58% of HAW participants; N = 1,047) and University of Southern California Study of Lifestyle and Women's Health (USC) (approximately 44% of USC participants, N = 1,996).

Institutional review board approval was obtained by the original studies, and all women had provided written informed consent.

Statistical Analysis

All data were self-reported using standardized in-person or phone interviews or self-completed questionnaires. The information collected reflected the time at each participant's reference date (i.e., date of diagnosis for cases, date of interview at the time of study enrollment for controls). We considered 10 risk factors whose associations with ovarian cancer have been well established in the literature. First-degree family history of ovarian cancer, tubal ligation, and NSAID use were evaluated as dichotomous yes/no or never/ever variables. Age at menarche was examined in age categories of <12, 12–14, and ≥15 years. Use of talc was categorized based on the area of application (i.e., genital or nongenital) with those who did not report using talc categorized as never users. Parity was grouped as nulliparous, 1, 2, and ≥3 births. BMI 1 year prior to the woman's reference date or 5 years prior for studies that did not ask for women's BMI 1 year prior was categorized as <18.5, 18.5–<25, 25–<30, and ≥30 kg/m^2. In addition, hormonal oral contraceptive use and breastfeeding were evaluated by total duration with categories of <1 (including never users), 1–<5, 5–<10, and ≥10 years for hormonal oral contraceptive use and never, <12, 12–<24, and ≥24 months for breastfeeding. We only considered postmenopausal HT use, hence we used age 50 as a proxy for age at menopause and only counted HT used at age 50 or later in our duration categories of never users (including those whose use was only before menopause [i.e. before age 50]), <5, 5–<10, and ≥10 years. This was done for estrogen-only therapy and estrogen-progestin therapy separately.

For most covariates and risk factors, the percentage of women missing data was minimal, ranging from 0.0% missing age to 4.4% missing family history of ovarian cancer (Supplementary Table 1, available online). The only exceptions were for NSAID use (31.4% missing) and talc use, which was not collected in the Danish study (the Malignant Ovarian Tumor Study [MAL]) and was missing in 5.0% of women in the Australian study (the Australian Ovarian Cancer Study [AUS]) and 41.4% in the US studies (Supplementary Table 1). Multiple imputation (*mice* package in R) was used to address data missingness, and 50 imputed datasets were generated. All variables in the dataset were initially considered for imputation, including those that were not used in the final models. The data were imputed separately for cases and controls and by geographic location (i.e., Australia, Denmark, the United States). The OCAC study was included as a predictor in the imputation for US studies.

All data were pooled, and logistic regression models were fit to assess the association between each factor and ovarian cancer risk overall and by histotype (where the numbers allowed) for women who had a history of endometriosis and women who did not, separately. None of the studies directly matched on the ovarian cancer risk factors evaluated although some did match on race/ethnicity (HAW and USC), neighborhood (USC), or age at reference date (AUS, the Connecticut Ovary Study [CON], the Diseases of the Ovary and Their Evaluation Study [DOV], HAW, the Hormones and Ovarian Cancer Prediction [HOP], MAL, the New England Case-Control Study of Ovarian Cancer [NEC], USC). Because studies have shown that unconditional logistic regression adjusting for matched factors improves precision when matching does not approximate unique matching pairs (e.g., matching on sibling) (18, 19), we adjusted for age (<40, in 5-year age groups to 74, ≥75 years), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, and other), highest level of education attained (<high school, high school graduate, some college, and ≥college graduate) as a proxy for neighborhood/socioeconomic status as well as OCAC study. The impact of the other 9 factors on each factor's association with ovarian cancer risk was then evaluated, and only those that changed the association of interest by ≥10% were included in the final models. Sensitivity analyses adjusting on a priori confounders (i.e., those associated with both the exposure of interest and outcome and not mediators) were also conducted.

The models for breastfeeding were fit among parous women only (n = 17,919). In addition, since the study population included both pre and postmenopausal women, the models for estrogen-only therapy and estrogen-progestin therapy use were restricted to postmenopausal women (n = 14,661). Only exclusive estrogen-only therapy and estrogen-progestin therapy use was considered, hence postmenopausal women who used both types (n = 700) or an unknown type of HT (n = 149) were excluded from these analyses; those excluded from the analyses because of use of both estrogen-only therapy and estrogen-progestin therapy or an unknown type of HT (n = 849) had a similar proportion of endometriosis as those included in the analysis

TABLE 1

Characteristics of the Ovarian Cancer Association Consortium studies included in the analysis.

Site	Study name	Study location	Time period	Data collection method	No. of cases (% with endometriosis)	No. of controls (% with endometriosis)	Mean age at enrollment (SD)	
							Cases	Controls
AUS (9)	Australian Ovarian Cancer Study	Australia	2002–2005	Self-completed questionnaire	1,336 (8.2%)	1,491 (5.8%)	59.2 (10.7)	55.9 (12.5)
CON (11)	Connecticut Ovary Study	Connecticut, US	1999–2003	In-person interview	388 (12.4%)	551 (9.4%)	59.3 (10.9)	53.1 (10.4)
DOV (12)	Diseases of the Ovary and Their Evaluation Study	Washington, US	2002–2009	In-person interview	1,137 (11.3%)	1,828 (8.0%)	56.2 (8.9)	56.4 (9.3)
HAW (13)	Hawaii Ovarian Cancer Study	Hawaii, US	1993–2008	In-person interview	698 (10.7%)	1,103 (6.6%)	57.0 (12.7)	55.1 (14.6)
HOP (14)	Hormones and Ovarian Cancer Prediction	Western Pennsylvania, Northeast Ohio, Western New York, US	2003–2009	In-person interview	717 (8.5%)	1,802 (7.2%)	60.2 (12.3)	58.3 (12.4)
MAL (10)	Malignant Ovarian Tumor Study	Denmark	1994–1999	In-person or phone interview	504 (1.4%)	1,553 (1.0%)	58.8 (10.8)	57.1 (11.3)
NEC (15)	New England Case-Control Study of Ovarian Cancer	New Hampshire, Eastern Massachusetts, US	1992–2008	In-person interview	1,472 (9.9%)	2,100 (7.8%)	55.4 (11.1)	53.6 (12.5)
UCI (16)	University of California, Irvine Ovarian Cancer Study	Orange County and San Diego County, California, US	1995–2005	Self-completed questionnaire	348 (17.5%)	569 (12.7%)	57.8 (12.0)	54.2 (12.3)
USC (17)	University of Southern California Study of Lifestyle and Women's Health	Los Angeles, California, US	1993–2010	In-person interview	1,900 (10.2%)	2,595 (6.7%)	57.3 (11.7)	54.4 (12.3)
				Total:	8,500 (9.8%)	13,592 (6.7%)	57.5 (11.3)	55.5 (12.1)

AUS = Australian Ovarian Cancer Study; SD = standard deviation; HAW = Hawaii Ovarian Cancer Study; USC = University of Southern California Study of Lifestyle and Women's Health; CON = Connecticut Ovary Study; DOV = Diseases of the Ovary and Their Evaluation Study; HOP = Hormones and Ovarian Cancer Prediction; MAL = Malignant Ovarian Tumor Study; NEC = New England Case-Control Study of Ovarian Cancer; UCI = University of California, Irvine Ovarian Cancer Study; USC = University of Southern California Study of Lifestyle and Women's Health; No. = number; SD = standard deviation.

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(n = 13,812) (9.4% and 7.5%, respectively). Because information on talc use was not collected in the study conducted in Denmark (MAL), the analyses for talc use were only based on the 8 OCAC studies in the United States and Australia.

Odds ratios (ORs) across the 50 imputed datasets were pooled using Rubin's rule to obtain a single point estimate (20). Confidence intervals (CIs) were calculated from pooled standard errors, which were derived from within and between imputation variances (20, 21). Likelihood ratio tests comparing models with and without interaction terms were conducted to generate *P* values for interactions to determine whether endometriosis statistically significantly modified any of the risk factor to ovarian cancer associations.

Among the 830 ovarian cancer cases with endometriosis, 329 were high-grade serous, 33 were low-grade serous, 32 were mucinous, 190 were endometrioid, 133 were clear cell, and the remaining 113 were other invasive, epithelial tumor types that were not classified as 1 of these 5 main histotypes, including mixed cell and Brenner tumors. As such, we had limited numbers to conduct meaningful histotype-specific analyses for most associations by endometriosis status, with the exception of the BMI 25–<30 kg/m^2 category.

All tests were two-sided, and *P* values that were ≤0.05 were considered statistically significant. The analyses were performed using R Studio version 1.3.1073.

RESULTS

The analyses included a total of 22,092 women across the 9 OCAC studies, the majority of whom were postmenopausal (n = 14,661). Among the 8,500 cases with ovarian cancer and 13,592 controls, 9.8% (n = 830) and 6.7% (n = 914) reported a history of endometriosis, respectively (Table 1). Overall, we did not find any statistically significant interactions between endometriosis and the 10 ovarian cancer risk factors considered in our analysis, although we did observe some qualitative differences by endometriosis status.

Although endometriosis did not statistically significantly interact with BMI (*P*-interaction = .51), among those with endometriosis, being overweight (i.e., BMI = 25–<30 kg/m^2) was associated with a 27% increased risk of ovarian cancer compared with those having a normal weight (i.e., BMI = 18.5–<25 kg/m^2) (OR = 1.27; 95% CI, 1.00–1.60), but showed no association for those without endometriosis (OR = 0.97; 95% CI, 0.91–1.05) (Table 2). An increased risk was also observed for those classified as obese (i.e., BMI = ≥30 kg/m^2), although there was little difference in the ORs for those with endometriosis (OR = 1.21; 95% CI, 0.94–1.57) vs. those without (OR = 1.13; 95% CI, 1.04–1.22) (Table 2). When we considered histotype, we observed a difference in the association between being overweight and risk of ovarian cancer across histotypes when comparing women with and without endometriosis, although none of the interactions were statistically significant (Supplementary Fig. 1, available online).

Having a first-degree family history of ovarian cancer was associated with an increased risk regardless of endometriosis status; however, the increased risk appeared greater for women without endometriosis than women with endometriosis (OR = 2.20; 95% CI, 1.88–2.57 vs. OR = 1.58; 95% CI, 0.97–2.57, respectively; *P*-interaction = .20) (Table 2). Genital talc use was also positively associated with risk for women with and without endometriosis, although its magnitude seemed to be greater for women with than women without (OR = 1.38; 95% CI, 1.04–1.84 vs. OR = 1.12; 95% CI, 1.01–1.25, respectively; *P*-interaction = .65) (Table 2). A similar pattern was observed for longer menopausal estrogen-only therapy use; the increased risk appeared greater for women with vs. without endometriosis, particularly for those who used estrogen-only therapy for ≥10 years (OR = 1.88; 95% CI, 1.09–3.24 vs. OR = 1.42; 95% CI, 1.14–1.76, respectively, *P*-interaction = .96) (Table 3). On the other hand, use of estrogen-progestin therapy was inversely associated with ovarian cancer risk among women with endometriosis, but not associated with risk among women without endometriosis (for 5–<10 years: OR = 0.64; 95% CI, 0.38–1.07 vs. OR = 0.98; 95% CI, 0.84–1.14, respectively; *P*-interaction = .57) (Table 3).

For breastfeeding, hormonal oral contraceptive use, parity, tubal ligation, age at menarche, and NSAID use, the magnitudes of their associations with risk of ovarian cancer did not appear to differ by endometriosis status (Tables 2 and 3). Overall, none of the results changed when sensitivity analyses were conducted adjusting for a priori confounders (Supplementary Tables 2 and 3, available online).

DISCUSSION

Endometriosis is a common gynecologic condition and a well-established risk factor for ovarian cancer (22). Given the previous work showing hysterectomy's association with ovarian cancer risk to differ by endometriosis status (6, 7), we examined the relationships of 10 other ovarian cancer risk factors, including BMI, talc use, first-degree family history of ovarian cancer, NSAID use, breastfeeding, hormonal oral contraceptive use, parity, tubal ligation, HT use, and age at menarche. To our knowledge, this is the first analysis that considers all of these well-established ovarian cancer risk factors when examining endometriosis' potential interactions with regard to the ovarian cancer risk.

Although we did not observe a statistically significant interaction between endometriosis and BMI, the higher risk associated with being overweight among women with endometriosis is interesting because endometriosis is considered an inflammatory disease (23) and adiposity contributes to a proinflammatory state (24). This was seen across histotypes, and a possible explanation may be related to inflammation. Because inflammation plays a role in the development of many cancers, including ovarian cancer (25), the increased risk observed specifically among women with endometriosis is plausible because overweight women with endometriosis may have higher levels of inflammation. Both endometriotic foci (26, 27) and adipose tissues (28) produce proinflammatory cytokines, including TNF-α, IL-1, and IL-6. These proinflammatory cytokines have been shown to increase the risk of ovarian cancer as they promote the synthesis of prostaglandins (3), which in turns inhibits cell differentiation and apoptosis (29), and enhances invasion and angiogenesis (30). This would also be in line with our observation of a higher risk associated with genital talc use for women with endometriosis since

TABLE 2

Associations between family history and lifestyle factors and ovarian cancer risk by endometriosis status.

	Without Endometriosis (7,670 cases, 12,678 controls)				With Endometriosis (830 cases, 914 controls)				
Risk Factor	**Cases[a]**	**Controls[a]**	**OR[b]**	**95% CI**	**Cases[a]**	**Controls[a]**	**OR[b]**	**95% CI**	***P*-interaction[c]**
BMI									.51
<18.5 kg/m^2	178	290	1.12	0.92–1.36	16	21	0.82	0.42–1.62	
18.5-<25 kg/m^2	3,554	6,267	1.00	—	403	475	1.00	—	
25–<30 kg/m^2	2,142	3,588	0.97	0.91–1.05	230	231	1.27	1.00–1.60	
≥30 kg/m^2	1,670	2,462	1.13	1.04–0.22 *P*-trend = .04[d]	172	186	1.21	0.94–1.57 *P*-trend = .06[d]	
Talc use[e]									.65
Never	2,172	4,137	1.00	—	220	323	1.00	—	
Nongenital use	1,391	1,909	0.76	0.49–1.19	162	140	0.83	0.39–1.77	
Genital use	827	1,304	1.12	1.01–1.25	79	106	1.38	1.04–1.84	
First-degree family history of ovarian cancer									.20
No	6,943	11,811	1.00	—	762	841	1.00	—	
Yes	397	309	2.20	1.88–2.57	41	30	1.58	0.97–2.57	
NSAID use									.50
Never	3,996	6,914	1.00	—	359	393	1.00	—	
Ever	1,130	2,007	0.90	0.78–1.04	169	196	0.85	0.63–1.13	

BMI = body mass index; CI = confidence interval; NSAID = nonsteroidal anti-inflammatory drug; OR = odds ratio.
[a] Numbers may not sum to total because of missingness.
[b] Models were fit in 50 imputed datasets, pooled using Rubin's rule, and adjusted for age at reference date, race/ethnicity, education level, and Ovarian Cancer Association Consortium (OCAC) study.
[c] *P* value for interaction using a likelihood ratio test.
[d] *P* value for trend was calculated by fitting the categorical variable as an ordinal variable.
[e] Models were fit among participants in studies conducted in Australia and the United States only.

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inflammation has been proposed as a possible biologic mechanism for talc's association with ovarian cancer (9).

Because endometriosis regresses after menopause and exposure to estrogen may reactivate endometriosis and stimulate carcinogenesis (31), we hypothesized that the association between estrogen-only therapy and ovarian cancer among women with endometriosis and women without endometriosis may differ. We did not observe endometriosis to statistically significantly interact with estrogen-only therapy, although longer use was associated with greater ovarian cancer risk among women with endometriosis, which is in line with estrogen's hypothesized role in endometriosis growth and ovarian cancer development. This difference in OR magnitude was not observed for short-term estrogen-only therapy use; however, this may be because estrogen-only therapy's effect on ovarian cancer has been shown to depend on the duration of use with substantial risk among long-term users (32). Interestingly, longer estrogen-progestin therapy use showed an inverse association for women with endometriosis, but there was no association with ovarian cancer risk for women without endometriosis. Some studies have shown that including a progestin component to an estrogen-only therapy regimen (i.e., estrogen-progestin therapy) may ameliorate some of the carcinogenic effects of estrogen when it comes to ovarian cancer risk (33, 34), and progestin therapy is often used to treat endometriosis (35). Information regarding the progestin included in the HT as well as endometriosis treatments would be relevant, although this information was unavailable.

A first-degree family history of ovarian cancer was associated with an increased ovarian cancer risk among women with and without endometriosis, although the magnitude of the association was greater for those who did not have endometriosis. It is unclear to us why this positive association may be greater for those without endometriosis. Studies have shown that high-grade serous, which is the most common histotype, is strongly associated with pathogenic variants in *BRCA1* and *BRCA2*, and this histotype is not associated with endometriosis (36). Endometrioid and clear cell ovarian cancer have also been shown to be associated with pathogenic variants in Lynch syndrome genes, and both histotypes are more common among women with endometriosis (36). Knowing the prevalence of these variants in women with and without endometriosis would be relevant, but to our knowledge, this has not been examined. However, at the same time, we acknowledge that the observed results may simply be due to chance given the small number of women with endometriosis and a family history of ovarian cancer.

A limitation of our study is that the information on endometriosis was based on self-report, and as such there could be misclassification. This misclassification would make the associations more similar when comparing women with and without endometriosis. However, it has been shown that self-reported endometriosis is reasonably accurate when compared with diagnosed endometriosis with at least 70% accuracy (37). An important strength of our study is our large sample size; we included over 22,000 women from various geographic regions, and of them, over 1,700 women had endometriosis. The only other study, to our knowledge, that has examined endometriosis' interactive effects with other ovarian cancer risk factors is the study by Modugno et al. (8), which included <400 women who self-reported a history

TABLE 3

Associations between hormonal and reproductive risk factors and ovarian cancer risk by endometriosis status.

	Without endometriosis (7,670 cases, 12,678 controls)				With endometriosis (830 cases, 914 controls)					
Risk Factor	**Cases[a]**	**Controls[a]**	**OR[b]**	**95% CI**	**Cases[a]**	**Controls[a]**	**OR[b]**	**95% CI**	***P*-interaction[c]**	**Risk factors adjusted**
Breastfeeding[d] (mo)									.91	Parity
Never	2,226	3,021	1.00	—	196	201	1.00	—		
<12	2,176	4,184	0.81	0.74–0.88	201	279	0.70	0.53–0.94		
12–<24	878	1,804	0.78	0.71–0.87	73	110	0.67	0.46–0.99		
≥24	547	1,510	0.60	0.53–0.68	42	78	0.60	0.38–0.96		
				P-trend < .001[e]				*P*-trend = .009[e]		
Duration of hormonal oral contraceptive use (y)									.91	
Never or <1	4,363	5,395	1.00	—	382	309	1.00	—		
1–<5	1,629	2,987	0.67	0.62–0.73	212	265	0.63	0.49–0.80		
5–<10	936	2,114	0.54	0.49–0.59	131	174	0.57	0.42–0.76		
≥10	714	2,163	0.38	0.35–0.42	103	162	0.47	0.34–0.64		
				P-trend < .001[e]				*P*-trend < .001[e]		
Parity									.40	Breastfeeding and tubal ligation
0 births	1,761	1,886	1.00	—	310	211	1.00	—		
1 birth	1,042	1,652	0.75	0.67–0.84	147	165	0.80	0.57–1.13		
2 births	2,229	4,236	0.63	0.57–0.70	216	279	0.75	0.54–1.02		
≥3 births	2,635	4,902	0.58	0.52–0.64	157	259	0.61	0.43–0.88		
				P-trend < .001[e]				*P*-trend = .009[e]		
Tubal ligation									.28	Parity and breastfeeding
No	6,444	9,666	1.00	—	714	667	1.00	—		
Yes	1,195	2,749	0.67	0.61–0.72	111	207	0.62	0.47–0.82		
Duration of estrogen-only therapy use[f] (y)									.96	
Never	4,274	6,367	1.00	—	372	425	1.00	—		
<5	425	699	0.97	0.83–1.13	60	69	0.83	0.52–1.32		
5–<10	218	268	1.17	0.95–1.45	36	34	1.23	0.71–2.12		
≥10	351	373	1.42	1.14–1.76	45	34	1.88	1.09–3.24		
				P-trend = .002[e]				*P*-trend = .05[e]		
Duration of estrogen-progestin therapy use[f] (y)									.57	Hormonal oral contraceptive use
Never	4,002	5,528	1.00	—	392	402	1.00	—		
<5	612	1,155	0.76	0.67–0.86	68	86	0.70	0.47–1.07		
5–<10	381	576	0.98	0.84–1.14	36	51	0.64	0.38–1.07		
≥10	255	414	0.93	0.76–1.12	17	22	0.68	0.33–1.39		
				P-trend = .06[e]				*P*-trend = .03[e]		
Age at menarche (y)										
<12	1,499	2,509	0.96	0.89–1.03	212	225	1.04	0.83–1.30	.76	
12–14	5,140	8,362	1.00	—	544	594	1.00	—		
≥15	981	1,731	0.89	0.81–0.97	73	90	0.89	0.63–1.25		
				P-trend = .35[e]				*P*-trend = .46[e]		

CI=confidence interval; OR=odds ratio.

[a] Numbers may not sum to total because of missingness.

[b] Models were fit in 50 imputed datasets, pooled using Rubin's rule, and adjusted for age at reference date, race/ethnicity, education level, Ovarian Cancer Association Consortium (OCAC) study, and other risk factors indicated in the "Risk factors adjusted" column.

[c] *P* value for interaction with endometriosis using a likelihood ratio test.

[d] Models were restricted to parous women only.

[e] *P* value for trend was calculated by fitting the categorical variable as an ordinal variable.

[f] Models were restricted to postmenopausal women only, excluding those who had ever used both estrogen-only therapy and estrogen-progestin therapy and those who used an unknown hormone therapy type.

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of endometriosis. Similar to the study by Modugno et al., we did not find any statistically significant interactions with hormonal oral contraceptive use, parity, and tubal ligation as well as observed ovarian cancer risk reductions of equal magnitude regardless of endometriosis status for all 3 factors. Although the type of hormonal oral contraception used likely varies between women with and without endometriosis, this information was unavailable.

Despite our large sample size, we had limited numbers to examine most associations by endometriosis status and histotype, and some risk factors have been shown to have histotype-specific effects. For example, studies have shown that BMI is associated with increased risk of endometrioid and low-grade serous ovarian cancer (38); estrogen-only therapy use is associated with increased risk of serous and endometrioid ovarian cancer (32); and genital talc use is associated with increased risk of serous, endometrioid, and clear cell ovarian cancer (39). It is possible that the differential associations that we observed by endometriosis status could partly be attributable to the histotype. However, when we examined the association between overweight and ovarian cancer by endometriosis status, we observed higher ORs among women with endometriosis regardless of the histotype.

In conclusion, our study is the first to examine endometriosis' interactive effects with 10 well-established ovarian cancer risk factors on risk of ovarian cancer. Most risk factors showed similar associations among women with and without endometriosis, and none of the interactions that we evaluated were statistically significant. However, there was some suggestion that the associations for BMI, genital talc use, and HT use may differ between women with and without endometriosis, which may be worth further exploring. A better understanding of the mechanisms underlying these findings is still needed, but regardless, our study provides some insight into the etiology of this complex disease.

Acknowledgments: The authors are grateful for all the contributions made by the researchers of the Ovarian Cancer Association Consortium. The authors express their deep appreciation to the women who participated in these studies as well as to the doctors, nurses, clinical and scientific collaborators, health care providers, and administrative staff who made this work possible.

Further, the Australian Ovarian Cancer Study (AUS) also acknowledges the cooperation of the participating institutions in Australia, and the contribution of the study nurses, research assistants, and all clinical and scientific collaborators. The complete AUS Study Group can be found at www.aocstudy.org. The AUS Study Group is thankful to all the women who participated in this research program. The Connecticut Ovary Study (CON) acknowledges the cooperation of the 32 Connecticut hospitals, including Stamford Hospital, in allowing patient access. The CON study was approved by the State of Connecticut Department of Public Health Human Investigation Committee. Certain data used in the CON study were obtained from the Connecticut Tumor Registry in the Connecticut Department of Public Health. The authors assume full responsibility for analyses and interpretation of the CON study's data.

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SUPPLEMENTARY TABLE 1. Characteristics of participants in the analysis by endometriosis status

	Without Endometriosis		With Endometriosis	
Characteristic	**Cases N (%)[a]**	**Controls N (%)[a]**	**Cases N (%)[a]**	**Controls N (%)[a]**
Age[b]	57.9 (11.4)	55.7 (12.2)	54.0 (9.72)	53.8 (10.3)
Race/ethnicity				
Non-Hispanic White	6406 (83.5%)	10913 (86.1%)	690 (83.1%)	802 (87.7%)
Hispanic White	343 (4.5%)	437 (3.4%)	21 (2.5%)	21 (2.3%)
Black	168 (2.2%)	242 (1.9%)	16 (1.9%)	15 (1.6%)
Asian	449 (5.9%)	561 (4.4%)	67 (8.1%)	44 (4.8%)
Other	279 (3.6%)	495 (3.9%)	32 (3.9%)	30 (3.3%)
Missing	25 (0.3%)	30 (0.2%)	4 (0.5%)	2 (0.2%)
Education level				
Less than high school	1352 (17.6%)	2112 (16.7%)	49 (5.9%)	53 (5.8%)
High school graduate	1845 (24.1%)	2766 (21.8%)	140 (16.9%)	182 (19.9%)
Some college	2048 (26.7%)	3213 (25.3%)	255 (30.7%)	260 (28.4%)
College graduate or above	2167 (28.3%)	4166 (32.9%)	355 (42.8%)	364 (39.8%)
Missing	258 (3.4%)	421 (3.3%)	31 (3.7%)	55 (6.0%)
Body mass index				
<18.5 kg/m^2	178 (2.3%)	290 (2.3%)	16 (1.9%)	21 (2.3%)
18.5-<25 kg/m^2	3554 (46.3%)	6267 (49.4%)	403 (48.6%)	475 (52.0%)
25-<30 kg/m^2	2142 (27.9%)	3588 (28.3%)	230 (27.7%)	231 (25.3%)
30+ kg/m^2	1670 (21.8%)	2462 (19.4%)	172 (20.7%)	186 (20.4%)
Missing	126 (1.6%)	71 (0.6%)	9 (1.1%)	1 (0.1%)
Breastfeeding				
Never	3987 (52.0%)	4907 (38.7%)	506 (61.0%)	412 (45.1%)
<12 months	2176 (28.4%)	4184 (33.0%)	201 (24.2%)	279 (30.5%)
12-<24 months	878 (11.4%)	1804 (14.2%)	73 (8.8%)	110 (12.0%)
24+ months	547 (7.1%)	1510 (11.9%)	42 (5.1%)	78 (8.5%)
Missing	82 (1.1%)	273 (2.2%)	8 (1.0%)	35 (3.8%)
Hormonal oral contraceptive use				
<1 year	4363 (56.9%)	5395 (42.6%)	382 (46.0%)	309 (33.8%)
1-<5years	1629 (21.2%)	2987 (23.6%)	212 (25.5%)	265 (29.0%)
5-<10 years	936 (12.2%)	2114 (16.7%)	131 (15.8%)	174 (19.0%)
10+ years	714 (9.3%)	2163 (17.1%)	103 (12.4%)	162 (17.7%)
Missing	28 (0.4%)	19 (0.1%)	2 (0.2%)	4 (0.4%)
First-degree family history of ovarian cancer				
No	6943 (90.5%)	11811 (93.2%)	762 (91.8%)	841 (92.0%)
Yes	397 (5.2%)	309 (2.4%)	41 (4.9%)	30 (3.3%)
Missing	330 (4.3%)	558 (4.4%)	27 (3.3%)	43 (4.7%)
Parity				
0 births	1761 (23.0%)	1886 (14.9%)	310 (37.3%)	211 (23.1%)

1 birth	1042 (13.6%)	1652 (13.0%)	147 (17.7%)	165 (18.1%)
2 births	2229 (29.1%)	4236 (33.4%)	216 (26.0%)	279 (30.5%)
3+ births	2635 (34.4%)	4902 (38.7%)	157 (18.9%)	259 (28.3%)
Missing	3 (0.0%)	2 (0.0%)	0 (0%)	0 (0%)
Tubal ligation				
No	6444 (84.0%)	9666 (76.2%)	714 (86.0%)	667 (73.0%)
Yes	1195 (15.6%)	2749 (21.7%)	111 (13.4%)	207 (22.6%)
Missing	31 (0.4%)	263 (2.1%)	5 (0.6%)	40 (4.4%)
Estrogen-only therapy use[c]				
Never	4274 (77.4%)	6367 (79.4%)	372 (69.7%)	425 (73.4%)
<5 years	425 (7.7%)	699 (8.7%)	60 (11.2%)	69 (11.9%)
5-<10 years	218 (3.9%)	268 (3.3%)	36 (6.7%)	34 (5.9%)
10+ years	351 (6.4%)	373 (4.6%)	45 (8.4%)	34 (5.9%)
Missing	257 (4.7%)	316 (3.9%)	21 (3.9%)	17 (2.9%)
Estrogen-progestin therapy use[c]				
Never use	4002 (72.4%)	5528 (68.9%)	392 (73.4%)	402 (69.4%)
<5 years	612 (11.1%)	1155 (14.4%)	68 (12.7%)	86 (14.9%)
5-<10 years	381 (6.9%)	576 (7.2%)	36 (6.7%)	51 (8.8%)
10+ years	255 (4.6%)	414 (5.2%)	17 (3.2%)	22 (3.8%)
Missing	275 (5.0%)	350 (4.4%)	21 (3.9%)	18 (3.1%)
NSAID use				
Never	3996 (52.1%)	6914 (54.5%)	359 (43.3%)	393 (43.0%)
Ever	1130 (14.7%)	2007 (15.8%)	169 (20.4%)	196 (21.4%)
Missing	2544 (33.2%)	3757 (29.6%)	302 (36.4%)	325 (35.6%)
Age at menarche				
<12 years	1499 (19.5%)	2509 (19.8%)	212 (25.5%)	225 (24.6%)
12-14 years	5140 (67.0%)	8362 (66.0%)	544 (65.5%)	594 (65.0%)
15+ years	981 (12.8%)	1731 (13.7%)	73 (8.8%)	90 (9.8%)
Missing	50 (0.7%)	76 (0.6%)	1 (0.1%)	5 (0.5%)
Talc use				
Never	2172 (28.3%)	4137 (32.6%)	220 (26.5%)	323 (35.3%)
Non-genital use	1391 (18.1%)	1909 (15.1%)	162 (19.5%)	140 (15.3%)
Genital use	827 (10.8%)	1304 (10.3%)	79 (9.5%)	106 (11.6%)
Missing	3280 (42.8%)	5328 (42.0%)	369 (44.5%)	345 (37.7%)
Total:	7670 (100%)	12678 (100%)	830 (100%)	914 (100%)

Note. The missing data for each characteristic were imputed in the analysis.
Abbreviations: NSAID=nonsteroidal anti-inflammatory drug.
[a] Number of cases/controls (% of cases/controls).
[b] Mean (standard deviation).
[c] Among postmenopausal women only.

SUPPLEMENTARY TABLE 2. Associations between family history and lifestyle factors and ovarian cancer risk by endometriosis status comparing main analysis to sensitivity analysis

	Main Analysis (adjusted for confounders using 10% criterion)				**Sensitivity Analysis (adjusted for *a priori* confounders)**			
	Without Endometriosis	**With Endometriosis**			**Without Endometriosis**	**With Endometriosis**		
	OR (95% CI)[a]	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**	**OR (95% CI)[a]**	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**
Body mass index			0.51				0.51	Menopausal status
<18.5 kg/m^2	1.17 (0.95-1.44)	0.82 (0.42-1.62)			1.16 (0.94-1.43)	0.84 (0.42-1.68)		
18.5-<25 kg/m^2	1.0	1.0			1.0	1.0		
25-<30 kg/m^2	0.98 (0.91-1.05)	1.26 (0.99-1.59)			0.97 (0.90-1.05)	1.24 (0.97-1.58)		
30+ kg/m^2	1.14 (1.05-1.24)	1.21 (0.94-1.57)			1.14 (1.05-1.23)	1.20 (0.92-1.57)		
First-degree family history of ovarian cancer			0.18				0.18	
No	1.0	1.0			1.0	1.0		
Yes	2.18 (1.85-2.57)	1.58 (0.97-2.57)			2.18 (1.85-2.57)	1.58 (0.97-2.57)		
NSAID use			0.50				0.50	
Never	1.0	1.0			1.0	1.0		
Ever	0.90 (0.78-1.04)	0.85 (0.63-1.13)			0.90 (0.78-1.04)	0.85 (0.63-1.13)		
Talc use			0.65				0.65	
Never	1.0	1.0			1.0	1.0		
Genital use	1.12 (1.01-1.25)	1.38 (1.04-1.84)			1.12 (1.01-1.25)	1.38 (1.04-1.84)		
Non-genital use	0.76 (0.49-1.19)	0.83 (0.39-1.77)			0.76 (0.49-1.19)	0.83 (0.39-1.77)		

Note. The above analyses are based on 8 Ovarian Cancer Association Consortium (OCAC) studies only. We cannot access the Malignant Ovarian Tumor Study's (MAL's) data at this time due to the European Union's General Data Protection Regulation.

Abbreviations: CI=confidence interval; NSAID=nonsteroidal anti-inflammatory drug; OR=odds ratio.

[a] Models were fit in 50 imputed datasets, pooled using Rubin's rule, and adjusted for age, race/ethnicity, education level, OCAC study, and other risk factors indicated in the "Risk Factors Adjusted" column.

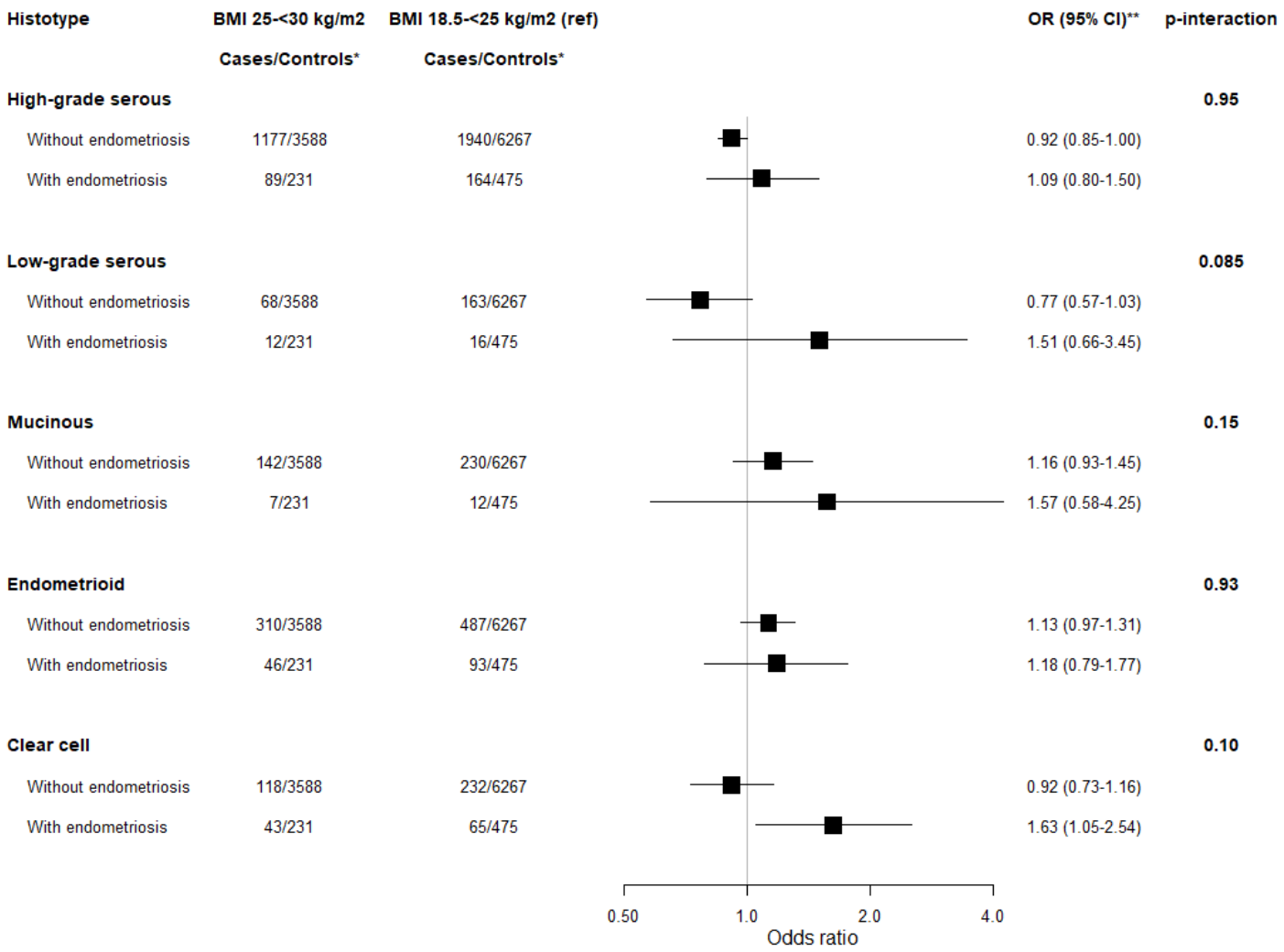
[b] P-value for interaction with endometriosis using a likelihood ratio test.

SUPPLEMENTARY TABLE 3. Associations between hormonal and reproductive risk factors and ovarian cancer risk by endometriosis status comparing main analysis to sensitivity analysis

	Main Analysis (adjusted for confounders using 10% criterion)				**Sensitivity Analysis (adjusted for *a priori* confounders)**			
	Without Endometriosis	**With Endometriosis**			**Without Endometriosis**	**With Endometriosis**		
	OR (95% CI)[a]	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**	**OR (95% CI)[a]**	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**
Breastfeeding[c]			0.91	Parity			0.91	Parity, hormonal oral contraceptive use
Never	1.0	1.0			1.0	1.0		
<12 months	0.81 (0.75-0.88)	0.70 (0.53-0.94)			0.82 (0.75-0.89)	0.69 (0.51-0.92)		
12-<24 months	0.78 (0.70-0.87)	0.67 (0.45-0.99)			0.78 (0.69-0.87)	0.64 (0.43-0.95)		
24+ months	0.59 (0.52-0.67)	0.59 (0.37-0.95)			0.57 (0.50-0.65)	0.55 (0.34-0.89)		
Hormonal oral contraceptive use			0.22				0.19	Age at menarche, parity, breastfeeding
Never or <1 year	1.0	1.0			1.0	1.0		
1-<5 years	0.67 (0.62-0.73)	0.63 (0.49-0.81)			0.71 (0.66-0.77)	0.65 (0.50-0.84)		
5-<10 years	0.54 (0.49-0.59)	0.56 (0.42-0.75)			0.55 (0.50-0.61)	0.56 (0.41-0.75)		
10+ years	0.38 (0.35-0.43)	0.47 (0.34-0.63)			0.37 (0.34-0.41)	0.45 (0.33-0.62)		
Parity			0.36	Breastfeeding, tubal ligation			0.22	Hormonal oral contraceptive use
0 births	1.0	1.0			1.0	1.0		
1 birth	0.75 (0.66-0.84)	0.80 (0.57-1.12)			0.66 (0.59-0.74)	0.64 (0.48-0.86)		
2 births	0.65 (0.58-0.72)	0.74 (0.54-1.02)			0.53 (0.48-0.58)	0.53 (0.41-0.69)		
3+ births	0.59 (0.52-0.65)	0.61 (0.43-0.88)			0.41 (0.38-0.45)	0.39 (0.29-0.52)		
Tubal ligation			0.22	Parity, breastfeeding			0.18	Parity, hormonal oral contraceptive use
No	1.0	1.0			1.0	1.0		
Yes	0.66 (0.61-0.72)	0.60 (0.46-0.80)			0.70 (0.65-0.77)	0.64 (0.49-0.85)		
Age at menarche			0.66				0.66	
<12 years	0.94 (0.87-1.02)	1.05 (0.83-1.32)			0.94 (0.87-1.02)	1.05 (0.83-1.32)		
12-14 years	1.0	1.0			1.0	1.0		
15+ years	0.91 (0.82-1.00)	0.91 (0.65-1.27)			0.91 (0.82-1.00)	0.91 (0.65-1.27)		

	Main Analysis (adjusted for confounders using 10% criterion)				Sensitivity Analysis (adjusted for *a priori* confounders)			
	Without Endometriosis	**With Endometriosis**			**Without Endometriosis**	**With Endometriosis**		
	OR (95% CI)[a]	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**	**OR (95% CI)[a]**	**OR (95% CI)[a]**	**p-interaction[b]**	**Risk Factors Adjusted**
Estrogen-only therapy use[d]			0.94				0.91	Hormonal oral contraceptive use
Never	1.0	1.0			1.0	1.0		
<5 years	1.05 (0.90-1.23)	0.85 (0.53-1.36)			1.08 (0.92-1.26)	0.86 (0.54-1.39)		
5-<10 years	1.20 (0.97-1.49)	1.23 (0.71-2.13)			1.25 (1.00-1.55)	1.27 (0.73-2.21)		
10+ years	1.48 (1.24-1.77)	1.95 (1.12-3.37)			1.51 (1.26-1.80)	2.09 (1.20-3.64)		
Estrogen-progestin therapy use[d]			0.67	Hormonal oral contraceptive use duration			0.67	Hormonal oral contraceptive use
Never	1.0	1.0			1.0	1.0		
<5 years	0.74 (0.65-0.84)	0.71 (0.47-1.08)			0.74 (0.65-0.84)	0.71 (0.47-1.08)		
5-<10 years	0.92 (0.78-1.07)	0.64 (0.38-1.08)			0.92 (0.78-1.07)	0.64 (0.38-1.08)		
10+ years	0.89 (0.74-1.08)	0.69 (0.33-1.41)			0.89 (0.74-1.08)	0.69 (0.33-1.41)		

Note. The above analyses are based on 8 Ovarian Cancer Association Consortium (OCAC) studies only. We cannot access the Malignant Ovarian Tumor Study's (MAL's) data at this time due to the European Union's General Data Protection Regulation.
Abbreviations: CI=confidence interval; OR=odds ratio.
[a] Models were fit in 50 imputed datasets, pooled using Rubin's rule, and adjusted for age at reference date, race/ethnicity, education level, OCAC study, and other risk factors indicated in the "Risk Factors Adjusted" column.
[b] P-value for interaction with endometriosis using a likelihood ratio test.
[c] Models were restricted to parous women only.
[d] Models were restricted to postmenopausal women only, excluding those who had ever used both estrogen-only therapy and estrogen-progestin therapy and those who used an unknown hormone therapy type.



SUPPLEMENTARY FIGURE 1. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between body mass index (25-<30 kg/m^2 compared to 18.5-<25 kg/m^2) and risk of ovarian cancer for women with and without endometriosis by histotype.
* Numbers may not sum to total due to missingness.
** Models were fit in 50 imputed datasets, pooled using Rubin's rule, and adjusted for age at reference date, race/ethnicity, education level, and Ovarian Cancer Association Consortium (OCAC) study.